

RELATIONSHIP BETWEEN SOME
PHYSICOCHEMICAL PROPERTIES
OF WHEAT PROTEINS AND BREADMAKING
QUALITY

by

FAKHRY HAFEZ GOBRAN

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ABSTRACT

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Relationship Between Some Physicochemical Properties of Wheat Proteins and Breadmaking Quality

Major Professor: Dr. W. Bushuk

Technological data were obtained for 26 wheat varieties of diverse baking quality grown at two locations in Western Canada. Highly significant correlations between remix loaf volume and protein content, and farinograph water absorption were obtained. Sedimentation value and dough development time were not significantly correlated with loaf volume and therefore are not reliable indices for prediction of bread-making quality. Intra- and inter-varietal variations in quality, as reflected by remix loaf volume per unit protein and BSI test values, were observed for the wheat samples investigated.

As found by other investigators, location of growth had no effect on the gliadin electrophoregram for all but one of the varieties. Minor differences were observed between the electrophoregrams of the samples from the two locations for the variety Glenlea. This was attributed to the unusually high tendency of this variety to outcross.

All of the high quality varieties had a characteristic doublet in the gliadin electrophoregram. All of the varieties that did not have

this doublet were of poor quality. However some varieties that had the doublet were also of poor quality. Accordingly, the doublet is not an exclusive marker of breadmaking quality.

Gliadin content (as determined by quantitation of the densitometric profile) appears to be related to baking potential; higher loaf volume varieties had less gliadin determined by this technique.

SDS-PAGE was used to examine both reduced and unreduced glutenins from the 26 wheat varieties grown at the two locations. No obvious differences in the electrophoregrams were observed that could be related to intervarietal differences in quality. As found by others, electrophoregrams of reduced glutenins were independent of the area of growth and seemed to be genetically controlled.

Gel-filtration profiles on Sephadex G-200 of the main wheat gluten fractions (gliadin and glutenin) of four wheat varieties of widely different baking potential did not show differences that can be related to differences in loaf volume. However, differences were observed in the gel-filtration profiles of the total protein extracted with AU solvent from three wheat varieties of different dough strength and baking potential. The strongest variety (Glenlea) contained less glutenin fraction than the two weaker varieties. Accordingly, the gel-filtration technique may be used as a tool to differentiate between very strong and very weak wheat varieties.

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I. INTRODUCTION

Research has shown that it is quite possible to have two flours milled from different varieties of bread wheat of the same class with the same protein content but of quite different baking quality. This difference is presumed to be due to differences in the chemical and physical properties of the proteins in the flour. The combination of these properties is generally referred to as "protein quality" for breadmaking.

As will be seen from the literature review that follows, breadmaking quality of wheat is governed primarily by a complex combination of physical and chemical properties of flour constituents. No single factor or component controls the quality of all bread wheats. Each constituent has some influence. The main controlling constituent may be different, depending on class or even variety of wheat.

As in many previous studies, the focus of the present study is on the protein component of the flour. Specifically, the study was designed to examine the proteins by several recently developed techniques such as polyacrylamide gel electrophoresis and gel filtration. The techniques were used to examine the proteins of 26 bread wheat varieties of widely different breadmaking quality, grown at two locations in Western Canada. The results obtained by the two analytical techniques were analysed in several different ways and the data so obtained correlated with breadmaking potential as reflected by a number of different technological tests. This thesis presents the results

obtained and discusses them in the context of the overall objective of this research - the chemical and physical nature of breadmaking quality of bread wheat.

II. LITERATURE REVIEW

A. General

Breadmaking potential of a flour milled from a bread wheat depends on a critical optimum combination of flour constituents, added ingredients, and processing parameters (Tipples 1977). Frequently, a deficiency in one group of factors can be corrected by adjustments in either of the other groups. Some practical examples are the addition of malted barley flour to improve the gassing power of the dough, addition of potassium bromate or other so-called flour improvers to optimize the rheological (flow) properties of the dough, adjustment of water absorption (amount of water needed to make an optimum dough) to balance the alpha-amylase activity, variation of mixing time to bring the gluten to optimum development, etc.

As the main ingredient of bread, flour contributes a number of constituents that play a significant role in determining the final quality of the bread (Pomeranz 1978). The key flour constituents are proteins (including enzymes), starch, lipids, and pentosans. Each constituent plays a definite role; the magnitude of its contribution can vary depending on whether we are comparing flours milled from wheat samples of the same variety, from samples of different varieties from a single class or from samples of varieties from different wheat classes.

For wheat varieties from the same class, differences in bread-making potential are due, primarily, to differences in the protein component. The key publication in this area is that of Finney and Barmore (1948). These workers showed that for varieties of hard red winter wheat, breadmaking potential (as measured by loaf volume) is directly related to the protein content of the flour. It should be noted that protein content of wheat samples of a specific variety can vary from about 7% to over 20%, depending on conditions of growth (soil fertility, moisture, temperature, etc.) (Finney et al. 1957; Shellenberger 1978).

Since the pioneering work of Finney and Barmore (1948), the linear relationship between protein content (for samples of one or similar varieties) and breadmaking potential has been found for other classes of wheat (Fifield et al. 1950; and Bushuk et al. 1969).

Finney and Barmore (1948) observed also that the slope of the loaf volume - protein content relationship depends on variety. That is, a unit of protein in one variety can contribute a different increment to the loaf volume than for another variety. The magnitude of this increment, obviously, depends on the intrinsic properties of the proteins that contribute to loaf volume of baked bread. The properties involved are many and complex; collectively they are referred to as "protein quality" for breadmaking (Tipples 1977; Pomeranz 1978). Furthermore, it is now known that those properties are

genetically controlled and are inherited in the progeny from the parents (Sozinov and Popereya 1979). Nevertheless, the quality can be affected by abnormal environment during growth (e.g. disease, moisture stress, temperature, sprouting damage, frost damage, etc.) (Bushuk 1977).

The scientific literature contains numerous publications on attempts to determine the nature (chemical and physical properties) of protein quality. The reader is referred to several excellent recent reviews on the subject (Kasarda et al. 1976; Tipples 1977; Kasarda et al. 1978; Pomeranz 1978; Bloksma 1978).

B. Flour Protein and Breadmaking Quality

Protein is the component of flour that accounts for a major proportion of the differences in baking potential of wheat samples. Differences between samples of the different varieties but of the same protein content are attributed to differences in "protein quality" (Pomeranz 1966).

The two most successful approaches to the study of protein quality have been the fractionation - reconstitution approach used extensively by Hoseney et al. (1969a, b,c) and more recently by others (MacRitchie 1973; Booth and Melvin 1979) and the solubility fractionation approach of Orth and Bushuk (1972).

The fractionation - reconstitution technique has been applied to a small number of flours, usually two, one of good and the other of poor baking quality. Using this technique Hosney et al. (1969c) showed that for the two flours examined, gliadin controlled loaf volume whereas glutenin was related to the mixing time required to bring the dough to optimum development.

The first comprehensive study of wheat protein solubility was done by Osborne (1907). He identified four fractions according to differences in solubility in a series of solvents. These fractions are albumin (water-soluble); globulin (salt-soluble); gliadin (soluble in 70% ethanol); and glutenin (insoluble in alcohol but soluble in dilute acid or dilute alkali).

The modified Osborne solubility fractionation (Chen and Bushuk 1970) was applied by Orth and Bushuk (1972) to flours of 26 wheat varieties grown at four locations to determine possible relationship between the proportions of the protein fractions and breadmaking quality. That study showed loaf volume was significantly negatively correlated with the amount of soluble glutenin and positively with the amount of insoluble residue protein.

Earlier protein fractionation studies related to breadmaking quality are worthy of mention. Pence et al. (1951) found that the protein that is soluble in 0.1%, pH 6.8 phosphate buffer was required for maximum performance of all glens in breadmaking of all wheats

except durum wheat. They also showed that the dialyzable portion of the buffer-soluble fraction produced a positive response in loaf volume and decrease in mixing time when added to gluten-starch blends. Later, Pence et al. (1954a) showed, on the basis of fractionation results for 32 flours, that there was no significant relationship between the amount of soluble protein (albumin and globulin) and loaf volume. The same workers (1954b) also showed characteristic differences in the relative amounts of individual electrophoretic components among the water-soluble proteins of durum, club, and common wheat flours. Bell and Simmonds (1963) used two different solvents to extract the soluble proteins from 26 wheat samples having a wide range of protein content. A positive correlation was found between the amount of the fraction that was soluble in 0.05 M formic acid and loaf volume. The same article reported a negative correlation between the amount of the fraction soluble in 0.01 M sodium pyrophosphate and loaf volume.

Another approach related to solubility fractionation was used by Pomeranz (1965). He showed that loaf volume was negatively correlated with the amount of protein extractable from flour with 3 M urea solution. Maes (1966) found a negative correlation between baking quality and the percentage of protein soluble in water. This work was later supported by Booth and Melvin (1979) which showed that soluble protein was responsible for the poor baking quality of a high yielding European wheat variety. Hoseney et al. (1969a) in their study of the role of water-soluble protein in baking quality, found that water-

soluble protein was not responsible for loaf volume differences, however, gas production during dough fermentation increased with increasing proportion of this fraction. The comprehensive study of Orth and Bushuk (1972) of 26 varieties grown at four locations showed that the amount of water soluble protein was not correlated with loaf volume.

There have been a number of studies on the role of the salt-soluble proteins (globulins) in breadmaking quality. Koenig et al. (1964) showed that long-mixing flours had more salt-soluble protein than short-mixing flours. On the other hand, Mullen and Smith (1965) and Smith and Mullen (1965) found that short- and long-mixing flours contained similar amounts of salt-soluble protein. This was later confirmed by Orth and Bushuk (1972).

There have been several studies of the role of gliadin in bread-making quality. The two most relevant studies in this regard are those of Hoseney et al. (1969c) and Orth and Bushuk (1972).

Using the fractionation-reconstitution technique with two flours, Hoseney et al. (1979c) concluded that the difference in loaf volume between bread flours was due to the gliadin fraction. On the other hand, the solubility fractionation study of Orth and Bushuk (1972) showed that the amount of gliadin in 104 flour samples (26 varieties grown at 4 locations) was not related to loaf volume.

The reports of Hoseney et al. (1969c) and Orth and Bushuk (1972) attribute somewhat different roles to glutenin in breadmaking quality.

The earlier study, based on two flours, concluded that glutenin was responsible for the differences in mixing requirements of different flours whereas the study of Orth and Bushuk (1972) showed that loaf volume was inversely related to the amount of glutenin and directly to the amount of insoluble residue protein. The results of Orth and Bushuk were supported by the more recent work of Huebner and Wall (1976) and MacRitchie (1978). However further work is needed to determine the reason for the discrepancy between the results of Hoseney et al. (1969c) and Orth and Bushuk (1972).

There have been many attempts to relate breadmaking quality of flour to the electrophoretic properties of its proteins. Elton and Ewart (1964) showed that there were differences in electrophoretic patterns of the gliadin and water-soluble proteins among several wheat varieties. They suggested that the observed differences may be related to differences in baking quality. However, it has been noted that the gliadin patterns of several flours of different quality were quite similar (Ewart 1966). Coulson and Sim (1964) used starch gel electrophoresis to examine the effect of the area of growth on the gliadin electrophoretic patterns for 34 common wheat varieties. No difference was observed for each variety; it was therefore concluded that the pattern was not affected by the area of growth. On the basis of these results, they suggested that the gliadin electrophoretic pattern could be used as an accurate marker for cultivar (variety) identification. These observations were later confirmed by Lee and Ronalds (1967), and

Zillman and Bushuk (1979b).

Huebner and Rothfus (1968) used sulfoethyl cellulose column chromatography and starch gel electrophoresis to study the gliadins of ten wheat varieties representing the five different classes grown in the United States. Varieties from different classes showed a greater variation than those from the same class. They also showed that some poor-baking quality wheats gave patterns that were similar to those of good-baking quality wheats.

An extensive electrophoretic study of the gliadins of 80 wheat varieties grown under different environmental conditions at different locations in the world was carried out by Doekes (1968). He concluded that the gliadin electrophoretic pattern was independent of growth conditions and was a genotypic characteristic of the variety. He did not observe any clear relationship between the electrophoretic pattern and baking quality. Wrigley (1970) examined gliadins of a number of wheat cultivars using a two-dimensional technique that combined gel electrofocusing and starch gel electrophoresis. He found considerable differences in the two-dimensional "fingerprints" among varieties. The patterns of samples of the variety Spica of different protein content were qualitatively constant.

Although research, so far, has shown that seasonal and environmental effects do not affect the gliadin patterns, however, significant reductions in band intensities, which reflect gliadin

content, were observed in samples of sprouted or overheated wheat (Ellis 1971). Orth and Bushuk (1972) studied the electrophoretic patterns of gliadins of 26 wheat varieties of diverse baking quality grown at four locations. Although intervarietal differences were observed, there was no obvious relationship between these differences and baking quality.

More recently, Baker and Bushuk (1978) examined the gliadin patterns of the second backcross of hexaploid wheat (Triticum aestivum L. em Thell) cv. Pitic 62 to cv. Neepawa using polyacrylamide gel electrophoresis. They observed that not all of the gliadin bands identified were controlled by single genes but that there were groups of bands that were inherited as a unit and controlled by a group of linked genes.

A recent comprehensive study has been carried out by the Soviet workers Sozinov and Popereleya (1979) on the use of genetically determined polymorphism of gliadin protein and its relationship to technological properties and baking quality. They used starch gel-electrophoresis to determine the chromosomes carrying loci coding for gliadin components in the F_1 and F_2 progeny from crosses of Chinese Spring with hard winter wheat varieties Odesskaya 3, 16, and 26. They found that the gliadin components were inherited in a linkage form and were controlled by allelic loci. They also showed that the presence of some chromosomal blocks was directly related to baking quality. For example, the progeny that had chromosomal block Gld IB1

(gliadin block of components I controlled by chromosome B1) were of superior baking quality. On the other hand, the presence of chromosomal block Gld IB3 was associated with inferior baking quality. On the basis of these studies, the Soviet workers were able to develop the ideal gliadin formula in terms of allelic blocks for maximum quality. They concluded that such information should be of great help to solve many problems of plant breeding and seed production.

The use of the gliadin electrophoretic pattern to predict technological quality has been more successful in the case of durum wheats. It has been shown (Zillman and Bushuk 1979b; Damidaux et al. 1980; Kosmolak et al. 1980) that the presence of band 42 in the gliadin electrophoretic pattern by PAGE was associated with poor gluten quality (for spaghetti cooking quality) whereas the presence of band 45 was an indicator for good gluten quality. Since the electrophoretic technique can be used to analyze as little as half a wheat kernel, it should be extremely useful for screening parents and grain of early generations in durum wheat breeding programs.

Glutenin, the protein of gluten that imparts toughness and strength to dough, is less amenable to electrophoretic analysis than gliadin because of its high molecular weight and low solubility. However, considerable progress has been made on the physical nature of this protein by the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS - PAGE) (Bietz and Wall 1972, 1973, 1975; Orth and Bushuk 1973b, c, 1974; Khan and Bushuk 1976, 1977, 1979a).

Using moving boundary electrophoresis, Jones et al. (1959) found no significant differences in the electrophoretic patterns of the glutenins of bread wheat varieties of different baking quality. Orth and Bushuk (1973c) studied reduced glutenins of tetraploid and hexaploid wheats using sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) to examine the relationship between glutenin subunits and breadmaking quality. They observed major differences in the electrophoretic patterns between the tetraploid and the hexaploid wheats. The latter contained high mol wt subunits which appeared to be related to breadmaking quality. However, no differences were found among the varieties of hexaploid wheats that could be related to differences in baking quality. The same workers (1973b) reported that the SDS-PAGE patterns of glutenin subunits cannot be used to characterize the baking potential of bread wheats, although there were differences between varieties. The patterns appeared to be genetically controlled and were not affected by the area of growth. These results were confirmed in general by Butaki and Dronzek (1979). Khan and Bushuk (1979a) examined unreduced glutenins of different wheat varieties using SDS-PAGE. They found two groups of proteins in the unreduced glutenin complex, one comprising low mol wt subunits that entered the gel and the other comprising high mol wt subunits which remained at the origin. They suggested that the association between these two groups of glutenin subunits may be important to breadmaking quality.

The most extensive study of the relationship of SDS-PAGE patterns of glutenins of bread wheat varieties and baking quality is that of Payne et al. (1979) from the Plant Breeding Institute in Cambridge. They examined the glutenins from the progeny of a cross between good and poor quality wheat varieties. They showed that glutenin subunit of mol wt of 145,000 daltons inherited from the good quality parent was essential for breadmaking quality. However, these workers could not generalize their findings to include Canadian hard spring wheat varieties.

The review of the literature presented above indicates that considerable work has been done on the electrophoretic properties of gliadin and glutenin. It is now established that the electrophoretic patterns of these proteins are genetically controlled. And, since the gross technological properties (e.g. baking quality) are, to a great extent, also genetically controlled, there should be an explicit relationship between electrophoretic patterns and baking quality. So far, this has not been confirmed. The major portion of the present study is devoted to this elusive relationship.

III. MATERIALS

The wheat samples used in this study are from the 1979 Uniform Quality Nursery grown at Lethbridge and Swift Current under the supervision of Dr. A.B. Campbell of the Winnipeg Research Station, Agriculture Canada. The 26 varieties, their pedigrees and origin are listed in Table 1. All samples were used to examine the effect of environment on the gliadin and glutenin electrophoretic patterns (electrophoregram). Eight varieties, grown at Lethbridge, were selected for their high or low loaf volume potential and used in the densitometric and chromatographic studies. These varieties are listed in Table 2. Milling and baking quality data for all the samples are tabulated in Appendix I. All chemicals used in this study were of reagent grade.

Table 1. Uniform Quality Nursery
Varieties, Year 1979

Variety or Cross	Parentage	Origin
Manitou	Thatcher x 61PI 170925 (Red Egyptian type) /3/ Canthatch //Thatcher x 7/Frontana	Canada
Neepawa	Thatcher x 7/Frontana//Thatcher x 6/Kenya Farmer /3/Thatcher x 2//Frontana/Thatcher	Canada
RL2520//Tc* 6/KF	Frontana//RL 2265/Redman	Canada
Glenlea	Pembina 2 x 1 BAGE//CB 100	Canada
Era	II = 50 = 10/4/Pembina/II = 52 = 329 /3/II = 53 = 38/III = 58 = 4//II = 53 = 546	United States
Sonalika	II53 = 388/ANDES//Pitic 62 SIB/3/LR 64	India
Sinton	Manitou/3/Thatcher x 6/Kenya Farmer //Lee x 6/Kenya Farmer	Canada
Chester		Canada
Kenya 321.BT.1.B.1	Australia 45C5/Kenya II 7A	Kenya
Mida/Cadet	Mercury/RL 625	United State
Saric 70/Neepawa	Bluebird 3	Mexico
Pavon sib	CM-8399-D-4M-2Y-2M-3Y-1M-0Y	Mexico
Tesopaco sib	BR69-1Y-3M-0Y	Mexico
JIT-35-2L		India
H-Ra ² F ₂	II-41593-1R-3M-1S-2M-0S	INIA/ Mexico

Table 1. Uniform Quality Nursery Varieties, Year 1979 - Continued

Variety or Cross	Parentage	Origin
Bulbul	Pi62 - Frond/Pi62 - Mazoe x Mxp 65 PK 2858 - 7a - 3a - 4a - 0a	Pakistan
M.J. INTA		Argentina
ND 560	Olaf/Butte	United States
ND 563	ND 507//Wic 271/Polk	United States
James (SD 2273)	Olaf/ND 510 -2	United States
SD 2355	Olaf/Neepawa	United States
Len (ND 543)	ND 499/3/JTN/RL 4205/WI 261	United States
Mn 70170	Waldron/Era	United States
NAPB NSH 183-74		Canada
NAPB NSH 1001-75		Canada
CT 790		Canada

Table 2. Loaf Volumes of Eight Varieties Selected to Represent High and Low Loaf Volumes

Variety ¹	Remix Loaf Volume (cc)
Saric 70/Neepawa	1045
Kenya 321.BT.1.B.1	1000
Chester	1050
Sinton	1070
H-Ra 2F_2	570
Tesopaco Sib	570
Sonalika	675
Glenlea	620

¹All grown at Lethbridge

IV. METHODS

A. Milling and Breadmaking Quality Tests

All of the technological data shown in Appendix I, except the remix loaf volume, were obtained by the Approved Methods of the American Association of Cereal Chemists (A.A.C.C. 1962). The remix loaf volume was obtained by the baking procedure described by Irvine and McMullan (1960). This baking test is a straight dough method which uses vigorous dough mixing in order to accentuate the differences between "weak" and "strong" flours. The method has been particularly useful for differentiating varieties in the Canadian hard red spring wheat breeding program.

B. Gliadin Polyacrylamide Gel Electrophoresis

The flat-bed apparatus and the method used are those of Bushuk and Zillman (1978). The gel and tank buffer recipes are given in Table 3. The only modification to the original method was the replacement of aluminum lactate by sodium lactate in the running buffer solution. The procedure is as follows. A 6% gel was prepared by dissolving 12 g acrylamide, 0.6 g bisacrylamide, 0.25 g ascorbic acid, 0.002 g ferrous sulfate, and 0.3 g sodium lactate (60%) in distilled water to yield 200 ml of total volume. A final pH of 3.1 was obtained by adding lactic acid. To polymerize the gel solution (after chilling for 20 min. at 1°C), 1 ml of 3% hydrogen peroxide

Table 3. Composition of Gel and Tank Buffer Solution

Amount for 200 ml solution		
Gel solution		
Acrylamide	12.0	g
N,N' - methylene bisacrylamide	0.6	g
Ascorbic acid	0.25	g
Ferrous sulfate	0.002	g
Sodium lactate (60%)*	0.3	g
Lactic acid	to pH	3.1
Catalyst solution		
Hydrogen peroxide, 3%	1.0	ml
Tank buffer solution		
Sodium lactate (60%)*	0.3	g
Lactic acid	to pH	3.1

*Syrup form, Fisher Scientific Co.

(catalyst) was added just before pouring the solution into the electrophoresis apparatus.

The gliadin solution for electrophoresis was prepared by extracting 0.5 g ground grain with three times its weight (1.5 ml) of 70% aqueous ethanol in a stoppered centrifuge tube. The mixture was vortexed periodically during 60 min. at room temperature. After centrifugation (10 min. at $20,000 \times g$) the clear supernatant was decanted and mixed with 2 ml of tank buffer. Powdered sucrose (about 30% W/V) was dissolved in the sample solution to increase the density and facilitate sample application to the slot in the electrophoresis gel. Methyl green (about 0.02 g) was added to the sample solution to serve as a tracking dye during electrophoresis. Just prior to the start of electrophoresis, 17 μ l of sample was deposited into each slot. The electrophoresis was carried out at a constant current of 120 mA. The starting voltage corresponding to this current was 460 volts which dropped to 350 volts by the end of the run. Total run time of 4.5 to 5 hours was required for good resolution.

On termination of electrophoresis, the gel was removed from the apparatus by lifting with a piece of acrylic plastic of appropriate size and stained for 48 hrs in staining solution containing 0.1 g Coomassie Brilliant Blue R (dissolved in 10 ml of 95% ethanol) in 250 ml of 12% trichloroacetic acid. The gel was destained for 24 hrs in destaining solution containing 12% trichloroacetic acid in distilled water. Following destaining, the gel was rinsed with

water and photographed on Kodak 5069 High Contrast Copy film. The film was developed with D19 developer. Printing was done on Kodak Ektamatic SC photographic paper using Kodak Ektamatic A10 activator and Kodak Ektamatic S30 stabilizer.

C. Scanning of Electrophoregram by Densitometer

An Ortec Model 4310 densitometer, operating in visible light mode, was used to obtain the analog absorbance data from a positive film transparency of the electrophoregram. The absorbance was corrected for background density of the transparency and recorded on a standard length of chart paper. The area on each electrophoregram profile was determined with a planimeter. This area was considered as an estimate of the total gliadin content in each sample (that entered the gel on electrophoresis) and arbitrarily taken as one unit of protein.

D. Protein Solubility Fractionation

The dissociating solvent 'AUC', containing urea (3M), acetic acid (0.1M), and cetyltrimethyl ammonium bromide (0.01M) was used in this study as recommended by Meredith and Wren (1966) because of its high protein extractability. The alcohol-pH precipitation method of Orth and Bushuk (1973a) was used for glutenin and gliadin purification. The procedure was as follows. AUC solution (86 ml) was added to a gluten ball, obtained from 5 g ground grain, and the suspension kept

overnight at 4°C. The suspension was centrifuged for 20 min. at 20,000 xg at room temperature. The supernatant usually had a lipid overlayer and for this reason the required volume of clear supernatant was removed with a Pasteur pipette. Ethanol was added to the supernatant to a concentration of 70% (V/V). The solution was stirred for 30 min. at room temperature, its pH adjusted to 6.6 with 2N sodium hydroxide, and stored overnight at 4°C. The precipitate which formed was separated by centrifugation for 20 min. at 20,000 xg at room temperature. The precipitate was then redissolved with AUC solvent and the glutenin was reprecipitated by 70% ethanol at pH 6.6 and separated by centrifugation as indicated above. Finally this glutenin was dispersed in 100 ml of 0.01 M acetic acid and dialysed against frequent changes of distilled water for 2 days at 4°C. Continuous mixing during dialysis was provided by magnetic stirring. Following the dialysis the retentate was frozen and freeze-dried to yield "purified" glutenin.

The combined supernatants (containing gliadin) from the first and second treatments with AUC were dialysed against distilled water for 2 days at 4°C and freeze-dried to yield "purified" gliadin. The freeze dried samples were stored in a refrigerator and used as required.

E. Determination of Protein Content of The AUC Extracts

After selection of AUC as the protein solvent for wheat proteins by Meredith and Wren (1966), some authors (Bushuk and Wrigley 1971; Butaki 1977) expressed concern about the use of micro-Kjeldahl method for nitrogen determination (in freeze dried protein fractions) because of the possibility of urea contamination of the proteins. In the present study, the micro-Kjeldahl method was used and values obtained were corrected for urea nitrogen by the method of Evans (1968). This method is based on the reaction of urea with acidic diacetyl monoxime and thiosemicarbazide during a short heating period. The analytical procedure was as follows. Stock standard solution was prepared by dissolving 1.712 g of dried urea in distilled water up to 1 litre total volume. To this solution was added 5 drops of concentrated sulfuric acid. The nitrogen content (urea nitrogen) of this solution is 800 mg per litre (nitrogen accounts for 28/60 of the weight of urea). A working standard was prepared by diluting 1 ml of the standard stock solution to 10 ml with 5% trichloroacetic acid (TCA). Chemical reagents that were used in this assay are shown in Table 4. Standard curve data for urea nitrogen determination are shown in Table 5 and the standard curve in Fig. 1.

Before the application of this assay to AUC extracts, it was applied to a standard protein (bovine serum albumin) and to wheat protein extracted with solvent that did not contain urea. All results showed that the bovine serum albumin and the wheat protein

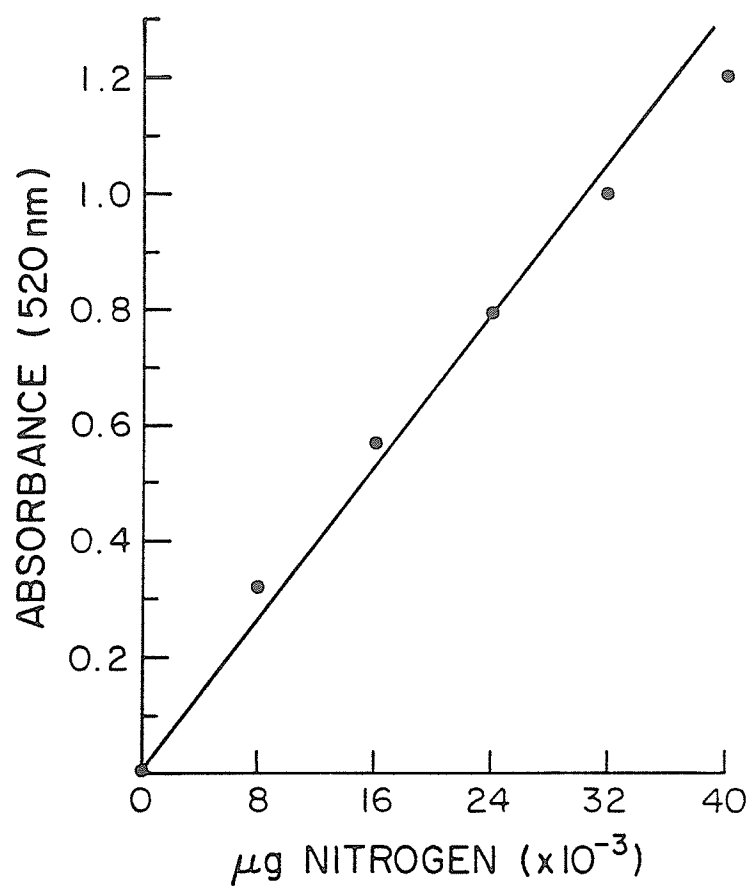
Table 4. Chemical Reagents for Urea Nitrogen Determination

Reagents	
Stock diacetyl monoxime (DAM)	
DAM	2.5 g
Distilled water	up to 100 ml
Stock thiosemicarbazide	
Thiosemicarbazide	0.25 g
Distilled water	up to 100 ml
DAM - thiosemicarbazide reagent	
DAM (stock)	24 ml
Thiosemicarbazide (stock)	10 ml
Distilled water	up to 100 ml
Acid reagent	
Concentrated sulfuric acid	80 ml
Phosphoric acid	10 ml
Ferric chloride	0.5 g
Distilled water	1000 ml
Color reagent	
DAM - thiosemicarbazide reagent	1 part
Acid reagent	5 parts

Table 5. Data for Standard Curve for Urea Nitrogen Determination

Tube	1	2	3	4	5	6
Working standard, ml	0.0	0.1	0.2	0.3	0.4	0.5
Urea nitrogen, μg	0.0	8	16	24	32	40
TCA (5%), ml	0.5	0.4	0.3	0.2	0.1	0.0
Color reagent, ml	5.0	5.0	5.0	5.0	5.0	5.0

Figure 1. Standard curve for urea nitrogen determination



do not interfere with the color reagent used for the urea assay.

Solutions of "purified" gliadin and glutenin were prepared for urea analysis by dissolving the protein in AUC solvent. Three methods of dialysis were applied before freeze drying the samples. The dialysis procedures were as follows:

1. AUC solutions were placed in dialysis tubing (exclusion limit - 14,000 daltons). Dialysis was carried out at 4°C against distilled water for 5 days. The dialysis solution (distilled water) was changed once every day during the dialysis period to ensure the removal of low molecular weight substances.
 2. This method of dialysis was the same as the first except that a magnetic stirrer was used to continuously mix the solution during dialysis. Total dialysis time in this case was two days during which time the dialysis solution was changed at least 10 times at regular intervals.
 3. Dialysis tubing containing the AUC - protein solutions were folded to form an annulus and placed inside a plastic tube attached to a rocker arm assembly. Distilled water was pumped at a constant flow around the dialysis tubing, causing the mechanism to periodically dip and empty before refilling. The total time for dialysis was 6 hr.
- At the end of each dialysis run, the dialysates were frozen and freeze-dried.

After the preparation of the samples by the three dialysis methods, urea nitrogen in the freeze-dried samples was determined as follows. Ten mg of dry sample was dispersed in 1 ml of 10% (TCA) and 1 ml of distilled water. The suspension was centrifuged at 10,000 xg for 10 min. at room temperature. To 0.5 ml of the clarified supernatant was added 5 ml of the color reagent (see Table 4). At the same time, a reagent blank was prepared by mixing 0.5 ml of 5% (TCA) and 5 ml of color reagent. The two solutions (sample and blank) were heated in boiling-water bath for 8 min. and then cooled to room temperature. Absorbancy of sample solution, corrected for that of the blank, was obtained at 520 nm on a Zeiss M4 QIII Spectrophotometer. The amount of urea nitrogen in the solution was determined from the standard curve in Fig. 1.

All data obtained showed a complete absence of urea nitrogen contamination in the samples obtained from the second and the third methods of dialysis. Results for samples obtained by the first method of dialysis showed that these contained 0.4 to 0.5 mg urea nitrogen per 100 mg dry sample. This amount of urea nitrogen would reflect an increase in protein content (after multiplying with the factor 5.7) of about 3%. On the basis of these results, the second method of dialysis was adopted to ensure the removal of all urea nitrogen by dialysis.

F. Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the procedure of Khan and Bushuk (1977) with one minor modification. Instead of the vertical apparatus, the flat bed apparatus, developed by Bushuk and Zillman (1978) for PAGE, was used. The procedure was as follows. For the 5% gel concentration, 11 g acrylamide and 0.27 g methylenebisacrylamide were dissolved in 200 ml of 0.125 M tris-borate buffer, pH 8.4, containing 0.1% SDS (W/V), 50 mg sodium sulfate, and 0.5 ml dimethylaminopropionitrile. To this solution, 5 ml of 2% ammonium persulfate (catalyst) were added just before pouring into the electrophoresis apparatus. After polymerization, the gel was pre-run for 20 min. at constant voltage (200 volts).

For SDS-PAGE, the protein samples were suspended in gel buffer solution with 1% SDS (W/V) at a concentration of 10 mg/ml and reduced with 1% mercaptoethanol (V/V) for 5 min. at 90°C and overnight at room temperature. Mercaptoethanol was omitted from the solution when reduction was not required. Centrifugation procedure was required at this stage to remove trace amounts of insoluble material present in the solution. Sucrose (about 10% W/V) was added to the supernatant to increase its density and bromophenol blue was added as a tracking dye.

Electrode buffer was 0.125 M tris-borate containing 0.1% SDS. Total time required for electrophoresis was 3.5-4 hr. at 200 volts; during this period the tracking dye migrates about 11 cm.

On termination of electrophoresis, the gel was rinsed with distilled water and stained overnight in staining solution comprising 1.6 g Coomassie Brilliant Blue, 800 ml ethanol (95%), 176 ml glacial acetic acid and 800 ml distilled water. The gels were destained in solution containing 25% methanol and 10% glacial acetic acid with gentle shaking until the background was clear (usually 5 days). Photographs were taken using Kodak 5069 High Contrast Copy film. The film was developed with D19 developer. Printing was done on Kodak Ektamatic SC photographic paper using Kodak Ektamatic A10 activator and Kodak Ektamatic S30 stabilizer.

G. Gel Filtration Chromatography

Sephadex G-200 was used in this study to determine the gel filtration profiles of the total extractable protein and protein fractions solubilized with AU solvent (aqueous solution of 0.1 N acetic acid and 3M urea). The filtration column was prepared as described by Khan and Bushuk (1979b). Sephadex G-200 was dispersed gently in distilled water for 24 hr. at room temperature and equilibrated for 3 days with AU elution solvent. The slurry was then deaerated by suction and poured into a 2.5 x 100 cm column and allowed to settle.

During conditioning and filtration, downward flow rate of eluent was controlled by a Mariotte-type container. Blue Dextran (mol wt

2,000,000) and tryptophan (mol wt 204) were used to determine the void and total elution volumes required for each run.

For chromatography, 70 mg of the freeze-dried protein sample was dissolved with 4 ml AU solvent and kept overnight at room temperature. For "total" protein extraction, 2 g sample of defatted ground grain was suspended in 15 ml AU solvent and kept overnight at room temperature. In both (freeze dried protein and ground grain) cases, the suspension was centrifuged for 30 min. at 20,000 xg at room temperature to remove trace amounts of undissolved materials. The same amount of protein (about 40 mg) was applied to the column in each run. The volume applied to the column was adjusted according to protein content to contain 40 mg protein. The column effluent (about 9 ml/hr) was collected in aliquots of 4.5 ml. Absorbance of each tube was determined at 280 nm on a Zeiss M4 QIII Spectrophotometer and used as the index of protein concentration in each tube to plot the gel filtration profile. Duplicate experiments were carried out for each elution profile. The agreement between duplicates was sufficiently close to permit averaging of the results.

V. RESULTS AND DISCUSSION

The results of this study are presented in five sections. Section A deals with statistical relationships between protein content and different quality parameters for the twenty-six varieties, each grown at two locations. Section B presents the results on the influence of growth area on the gliadin electrophoretic pattern (electrophoregram) and on the relationship between the distribution of gliadin components determined by polyacrylamide gel electrophoresis (PAGE) and breadmaking quality. Section C deals with the effect of location of growth on the densitometric profile of the gliadin electrophoregram and the relationship of the densitometric data and breadmaking quality. Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) results for reduced and unreduced glutenins of the 26 cultivars are presented in Section D which covers also the influence of location of growth on the SDS-PAGE patterns. Finally, Section E deals with the relationship between gel filtration profiles of total protein and protein fractions and breadmaking quality.

A. Protein Content and Breadmaking Quality

The technological data discussed in this section are tabulated in Appendix I. The correlation coefficients between protein content and selected parameters are presented in Table 6 (Lethbridge), Table 7 (Swift Current) and Table 8 (both stations). In the discussion

Table 6. Correlation Matrix of Five Quality Parameters
for Varieties Grown at Lethbridge

	Quality Parameter	1	2	3	4	5
1	Flour protein	1.0				
2	Sedimentation value	0.67**	1.0			
3	Farinograph absorption	0.54**	0.18	1.0		
4	Dough development time	0.45*	0.56**	0.22	1.0	
5	Remix load volume	0.59**	0.47*	0.66**	0.39*	1.0

** Significant at the 1% level

* Significant at the 5% level

Table 7. Correlation Matrix of Five Quality Parameters
for Varieties Grown at Swift Current

	Quality Parameters	1	2	3	4	5
1	Flour protein	1.0				
2	Sedimentation Value	0.62**	1.0			
3	Farinograph absorption	0.51**	0.06	1.0		
4	Dough development time	0.36*	0.50**	0.23	1.0	
5	Remix loaf volume	0.45*	0.31	0.60**	0.31	1.0

** Significant at the 1% level

* Significant at the 5% level

Table 8. Correlation Matrix of Five Quality Parameters
for Varieties Grown at Lethbridge and Swift
Current

	Quality Parameters	1	2	3	4	5
1	Flour Protein	1.0				
2	Sedimentation Value	0.67**	1.0			
3	Farinograph absorption	0.55**	0.17	1.0		
4	Dough development time	0.42**	0.52**	0.26*	1.0	
5	Remix loaf volume	0.51**	0.39**	0.59**	0.33**	1.0

** Significant at the 1% level

* Significant at the 5% level

that follows "highly significant" correlation, indicated by the standard double asterisk superscript, indicates 1% level of significance. Significance at the 5% level will be referred to as "significant" and indicated by a single asterisk superscript.

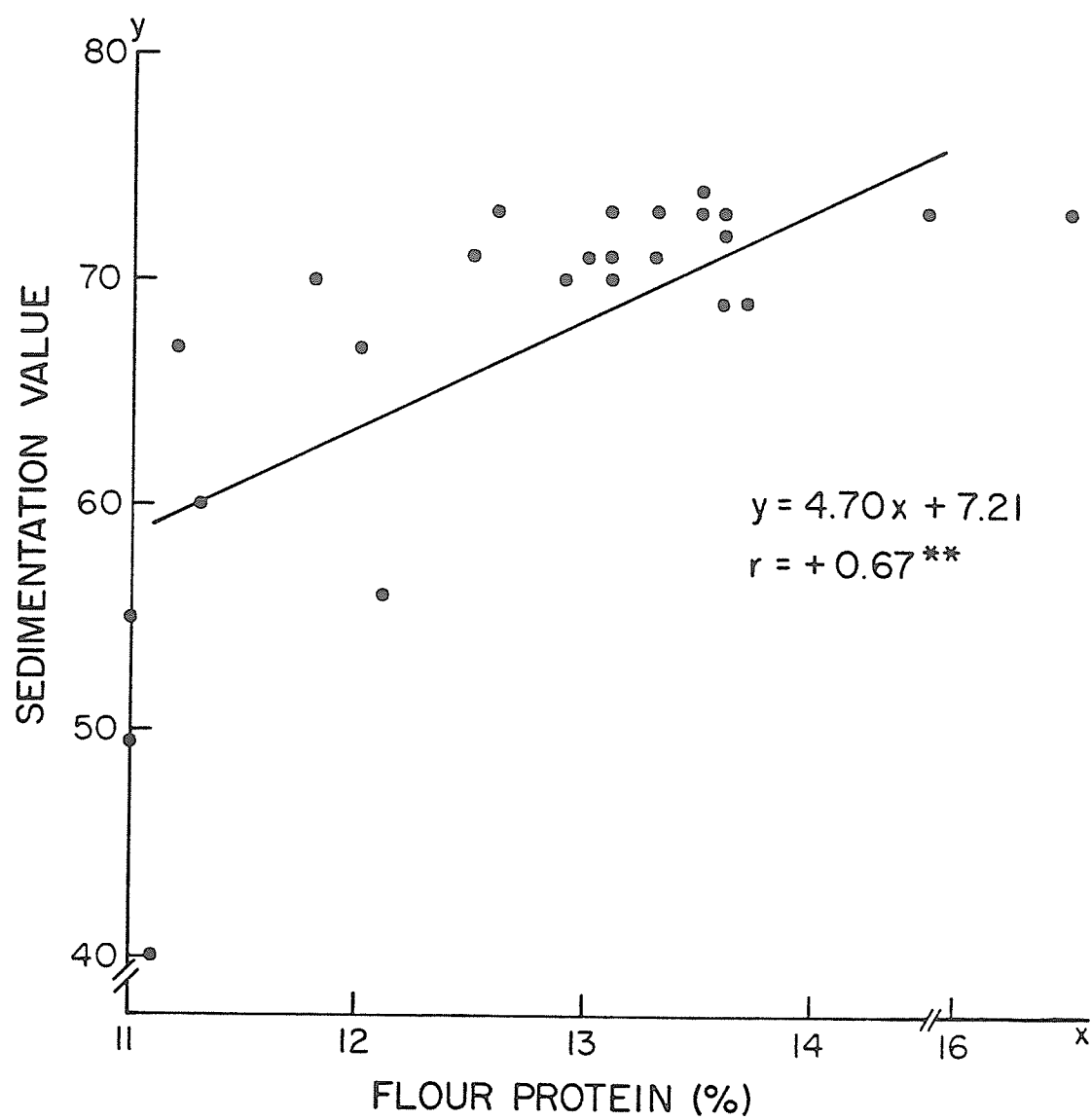
1. Sedimentation Value

The Sedimentation Value is the volume of a given amount of flour that has been allowed to swell in lactic acid solution and settle for a constant amount of time. It is used widely as a quality screening test in breeding programs.

In the present study, highly significant correlations were obtained between sedimentation value and protein content for the two sets of data analyzed individually or as a single population. The range of protein content in the samples analyzed is of sufficient width to make the statistical analysis valid. These results are in agreement with published information (Orth et al. 1972; Fowler and De La Roche 1975).

The scatter diagram (Figure 2) indicates relatively wide variability in sedimentation values at constant protein. This is borne out by relatively high standard deviation (± 6.49). The variability observed here was considerably greater than those obtained for samples of the same variety but of different protein content and reflects the wide variability in "protein quality" among the samples selected for this study. It is assumed, perhaps unjustifiably, that

Figure 2. Relationship between sedimentation value and flour protein content for varieties grown at Lethbridge



interclass variability in starch damage (which affects Sedimentation value) is minimal for the group of varieties used in the present study. These results (and others presented later) indicate that the wheat varieties selected for this study are suitable in terms of the objectives. The three correlation coefficients for protein and sedimentation value are: Lethbridge, $r = + 0.67^{**}$; Swift Current, $r = + 0.62^{**}$ and total population, $r = + 0.67^{**}$.

2. Farinograph Absorption

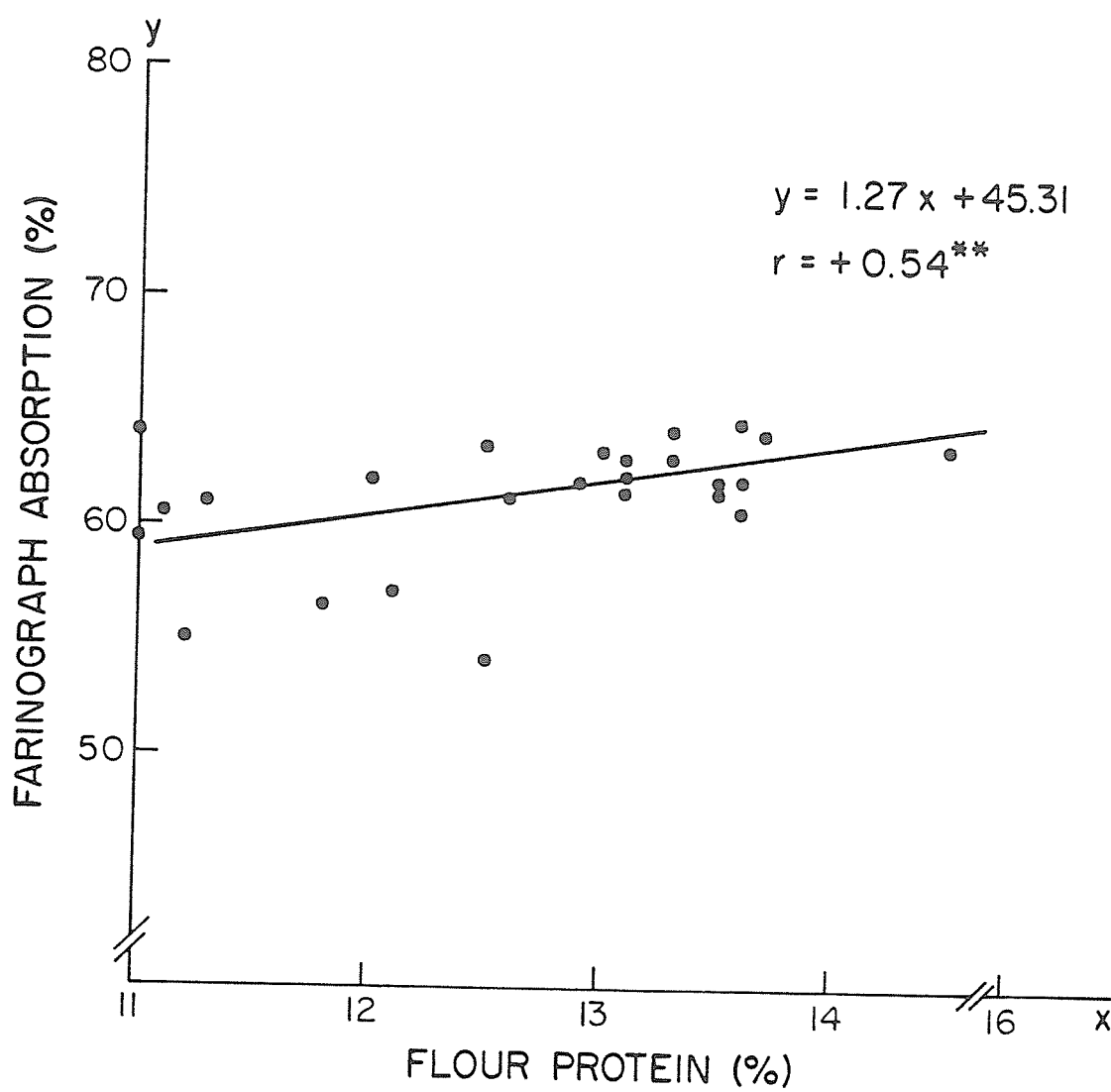
Farinograph absorption, which indicates the amount of water that must be added to the flour to produce an appropriate bread dough, was also highly significantly correlated with protein content. The r values are: Lethbridge, $r = + 0.54^{**}$; Swift Current, $r = + 0.51^{**}$ and total population, $r = + 0.55^{**}$. As in the case of the Sedimentation Value - protein relationship, the standard deviation for the absorption-protein correlation was quite high (± 2.48 ; Figure 3), indicating that "protein quality" contributes significantly to water absorption in addition to protein content. The results reported here are consistent with published work in this area for widely different varieties (Orth et al. 1972; Fowler and De La Roche 1975).

3. Dough Development Time

Dough development time, measured with the Farinograph, is technologically important in that it is an index of the energy (or mixing time) that is required to produce an appropriate bread dough. This study showed that dough development time is not highly significantly

Figure 3. Relationship between farinograph water absorption and protein content for varieties grown at Lethbridge





correlated with protein content in both locations (Lethbridge $r = + 0.45^*$; Swift Current $r = + 0.36^*$).

4. Remix Loaf Volume

The "remix loaf volume" is considered to be the all-inclusive index of baking quality of Canadian wheats (Irvine and McMullan 1960). Generally it is significantly correlated with protein content. In the present study a highly significant correlation was obtained for the Lethbridge samples ($r = + 0.59^{**}$; Figure 4) and for total population ($r = + 0.51^{**}$). For the Swift Current samples the correlation was significant but at the 5% level. The standard deviation for the Swift Current samples, (± 142), was higher than the Lethbridge samples (± 120). The reason for the higher standard deviation for the Swift Current results was not investigated.

The data in Tables 6, 7 and 8 show that loaf volume is highly significantly correlated with farinograph absorption (Figure 5) but the correlation with Sedimentation Value and dough development time was not significant at 1% level. The lack of significant correlation between remix loaf volume and sedimentation value questions the universal application of the Sedimentation Value for screening wheat populations in breeding programs for breadmaking quality. Indeed, the Sedimentation Value has not been particularly useful in Canadian programs and therefore is not extensively applied in Canada.

Two types of information, derived from the remix loaf volume, have been used in an attempt to obtain explicit information on "protein

Figure 4. Relationship between remix loaf volume and protein content for varieties grown at Lethbridge

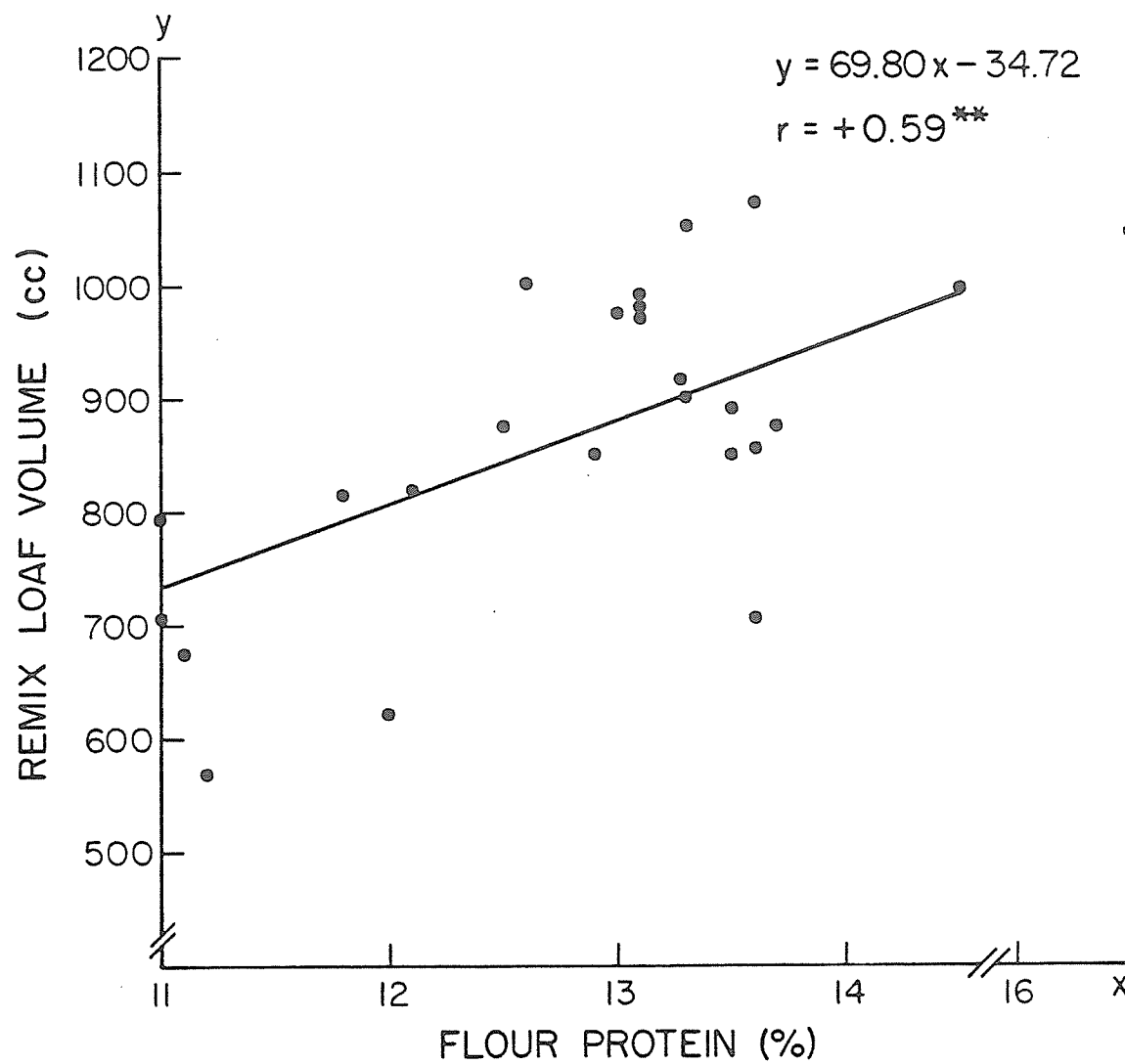
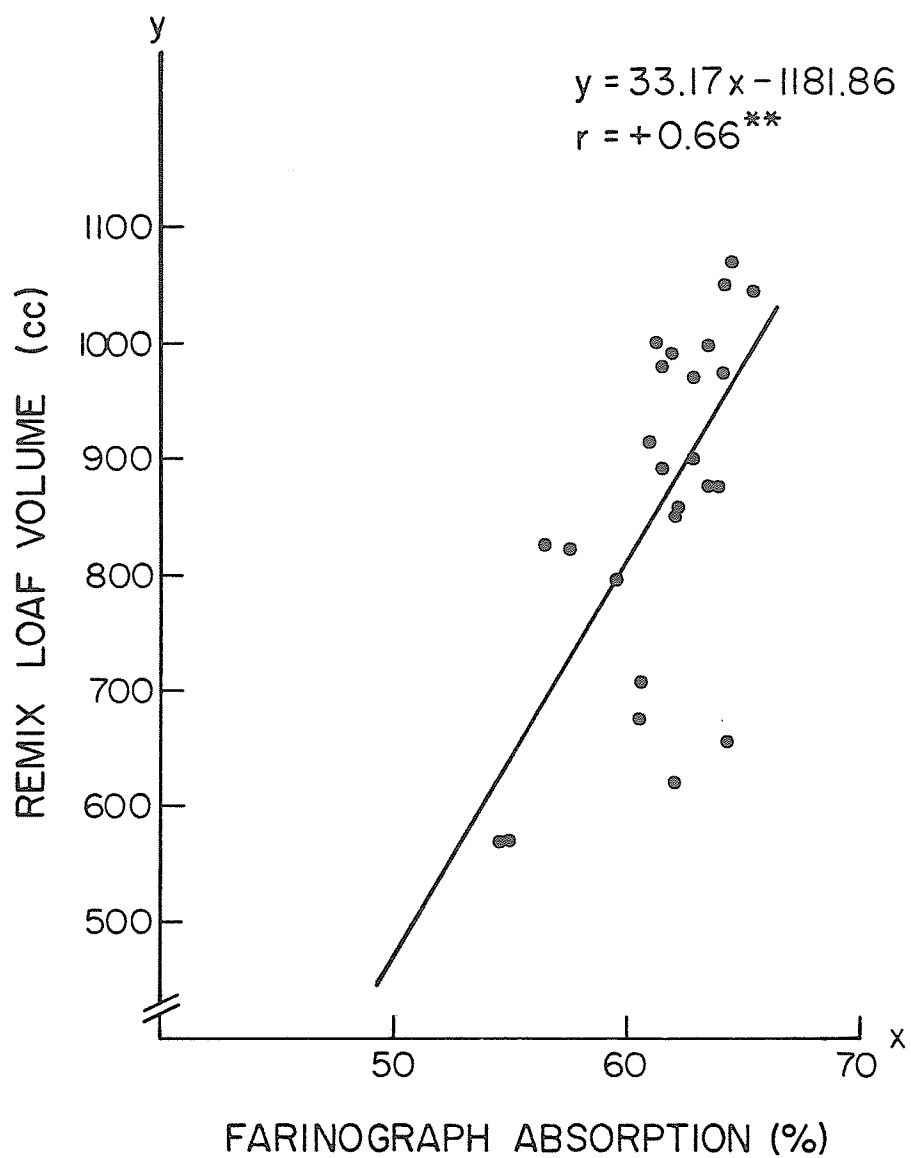


Figure 5. Relationship between remix loaf volume and
farinograph water absorption for varieties
grown at Lethbridge



quality" for breadmaking. These are: 1) loaf volume per unit protein used by Orth et al. (1972), and 2) the baking strength index (BSI) used by Tipples and Kilborn (1974). The BSI attempts to combine both loaf volume and protein content into a single index that reflects "protein quality". It is not used outside Canada.

Loaf volume per unit protein data (Table 9) show that this quality parameter depends on both genotypic and environmental factors. Furthermore, the differences between the stations for each variety are both positive and negative, indicating that genotype environment interaction contributes to this parameter.

The BSI values (Table 10) are consistent with the well known fact that protein "quality" contributes substantially to breadmaking potential. In the Canadian system, Neepawa is considered to be minimum quality standard in terms of BSI.

The data of Table 10 show several interesting anomalies in addition to the consistently high values for Canada Western red spring varieties and the extremely low value for the C.U. (Canada Utility) variety Glenlea. For example, the high yielding U.S. variety Era, which consistently has low protein content because of its high yielding capacity, has the highest BSI value. This point is worthy of note by Canadian wheat breeders. On the other hand, the variety Saric 70/Neepawa, has a relatively low BSI but still gave a high loaf volume because of its exceptionally high protein content. A comparative study of the

Table 9. Loaf Volume per Unit Protein for Twenty-Six Varieties from Two Stations

Variety	Loaf volume per unit protein (cc./%)*	
	Swift Current	Lethbridge
Manitou	54.7	61.1
Neepawa	56.4	60.0
RL 2520//Tc* 6/KF	57.5	60.8
Glenlea	36.2	41.3
Era	54.0	65.6
Sonalika	48.9	47.6
Sinton	52.8	63.5
Chester	55.7	64.0
Kenya 321.BT.1.B.1	62.0	63.3
Mida/Cadet	60.7	60.4
Saric 70/Neepawa	54.6	52.3
Pavon Sib	62.9	56.2
Tesopaco Sib	32.2	40.9
JIT-35-2L	51.0	55.6
H-Ra ² F ₂	27.0	36.1
Bulbul	57.5	50.1
M.J. INTA	43.3	50.8
ND 560	56.3	50.8
ND 563	46.5	51.4
James (SD 2273)	58.1	42.1
SD 2235	60.4	50.8
Len (ND 543)	54.2	53.8
Mn 70170	59.4	54.7
NAPB NHS 183-74	61.6	56.8
NAPB NHS 1001-75	58.5	57.0
CT 790	53.5	53.2

*Based on % protein on a dry basis

Table 10. Flour Protein, Remix Loaf Volume and Baking Strength Index for the Samples Grown at Lethbridge and Swift Current

Variety	Lethbridge			Swift Current		
	Flour Protein (%)	Remix Loaf Volume (cc)	BSI*	Flour Protein (%)	Remix Loaf Volume (cc)	BSI*
Manitou	13.1	980	114	13.8	915	101
Neepawa	13.1	970	113	13.7	925	103
RL 2520//Tc* 6/KF	13.0	975	114	13.3	930	107
Glenlea	12.0	620	79	13.2	585	68
Era	11.3	915	125	12.3	835	104
Sonalika	11.1	675	94	11.5	710	95
Sinton	13.6	1070	120	13.6	890	100
Chester	13.3	1050	120	14.0	945	102
Kenya 321.BT.1.B.1	12.6	1000	121	13.3	995	114
Mida/Cadet	13.1	990	115	14.0	1030	112
Saric 70/Neepawa	16.5	1045	95	15.9	1060	100
Pavon sib	12.5	875	107	13.0	1010	119
Tesopaco sib	11.2	570	79	12.2	490	62
JIT-35-2L	14.5	995	104	14.4	890	94
H-Ra ² F ₂	12.5	570	70	12.9	440	52
Bulbul	11.0	705	99	12.3	875	109
M.J. INTA	12.9	850	101	13.7	750	83
ND 560	13.5	850	96	13.7	950	105
ND 563	13.6	855	96	14.4	805	85
James (SD 2273)	13.6	705	79	13.6	945	106
SD 2355	13.7	875	97	13.7	1005	112
Len (ND 543)	13.3	900	103	13.6	895	100
Mn 70170	12.1	820	104	12.8	925	110
NAPB NHS 183-74	11.8	825	107	12.8	945	113
NAPB NHS 1001-75	11.0	795	112	12.2	885	111
CT 790	13.5	890	100	14.3	915	97

$$*BSI = \frac{\text{Loaf Volume} \times 100}{(\text{Flour protein} \times 70) - 58}$$

proteins of Era and Saric 70/Neepawa would be extremely interesting.

The above discussion pertains to the data for the Lethbridge samples. The data for the Swift Current samples demonstrate another interesting point. While these samples were generally higher in protein content than the Lethbridge samples, the BSI values for more than half of the samples were substantially lower, indicating a lower protein "quality". More data are needed to determine if this apparent effect of environment is real.

B. Gliadin Electrophoresis Results

1. Effect of Environment

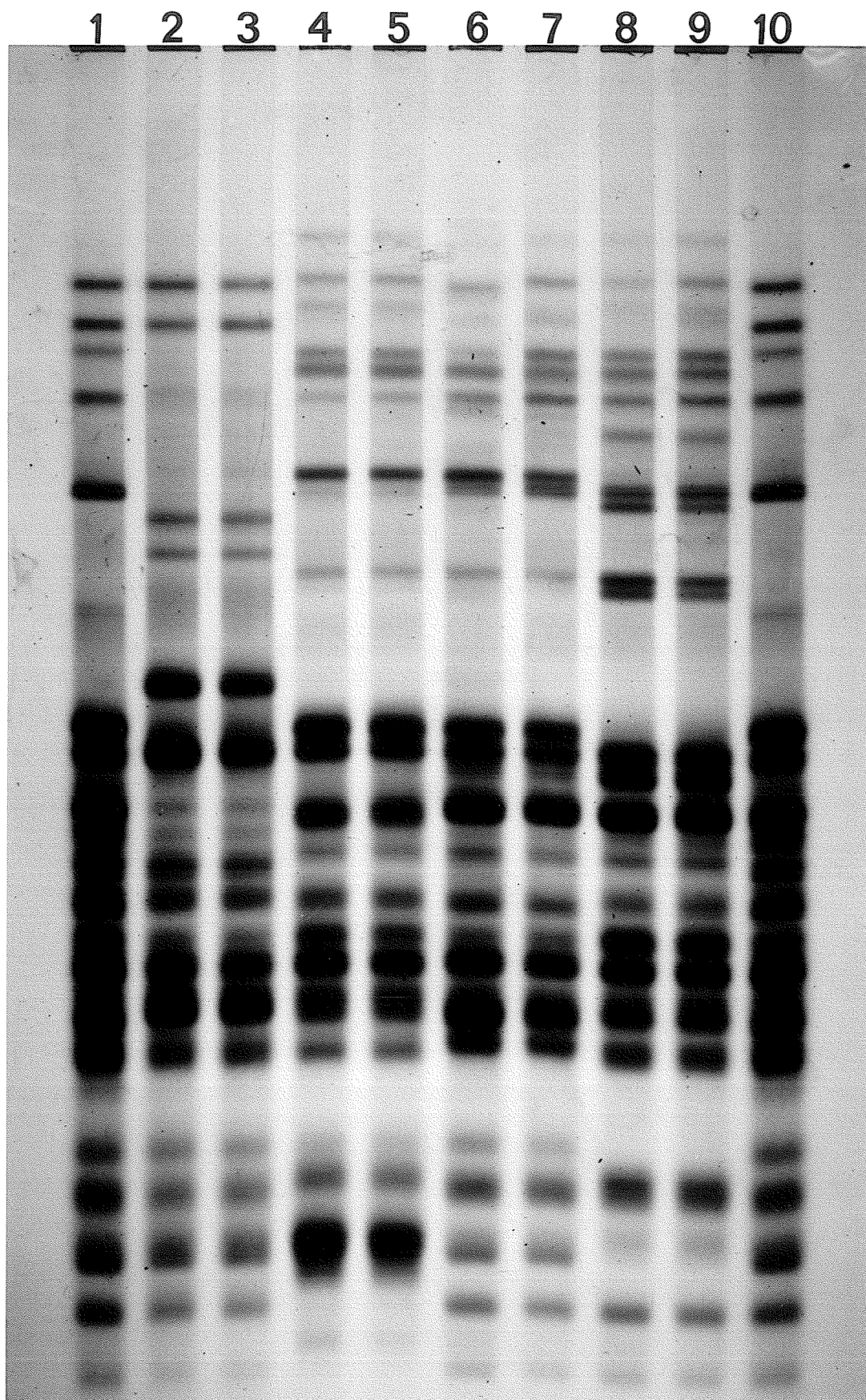
While it is well documented that the gliadin electrophoregram is a genotypic characteristic that is not affected by environment (Zillman and Bushuk 1979a and references therein) it was necessary to examine the samples from both stations to ensure that they are accurately identified. Typical results for four randomly selected varieties are shown in Figure 6. In each electrophoresis experiment patterns 1 and 10 were for the variety Marquis which has been adopted as the reference variety. The electrophoregrams for the remaining varieties used in this study are shown in Appendix II.

First of all, the results of Figure 6 show that sodium lactate can be freely substituted for aluminum lactate in the running buffer solution without any alteration to the electrophoregram. The pattern

Figure 6. Gliadin electrophoregrams of wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Figure in parentheses is the protein content
(N x 5.7, 14% m.b.)

- | | | | |
|-------------|------|-------------------------|------|
| 1. Marquis | | 6. RL2520//Tc*6/KF (SC) | |
| 2. Sonalika | (SC) | (13.9%) | |
| 3. Sonalika | (L) | 7. RL2520//Tc*6/KF (L) | |
| 4. Glenlea | (SC) | (13.8%) | |
| 5. Glenlea | (L) | 8. Neepawa | (SC) |
| | | (14.1%) | |
| | | 9. Neepawa | (L) |
| | | (13.9%) | |
| | | 10. Marquis | |



for the Marquis reference shown here is the same as that obtained by Bushuk and Zillman (1978) using the traditional aluminum lactate buffer. (This was also confirmed in the present study but the results are not shown.) As indicated in the Materials section, the reason for the substitution is the wider availability of sodium lactate in pure form.

Secondly, the two (two stations) electrophoregrams for each variety are identical. This is consistent with published information and confirms that the two samples are indeed of the same variety.

Thirdly, since some of the samples for each variety differed in protein content, the results presented here are consistent with the published information on the independency of the electrophoregram on protein content over the range of variability encountered under commercial production conditions. It has been shown (Zillman and Bushuk 1979a) that the electrophoregram of samples grown under conditions where there was an extreme lack of nitrogen, which leads to very low grain protein content, did not show some of the faint bands.

Finally, there is considerable variability among the electrophoregrams of the varieties examined in this study. Accordingly, their use in the present study on the relationship between breadmaking quality and electrophoretic properties, is justified.

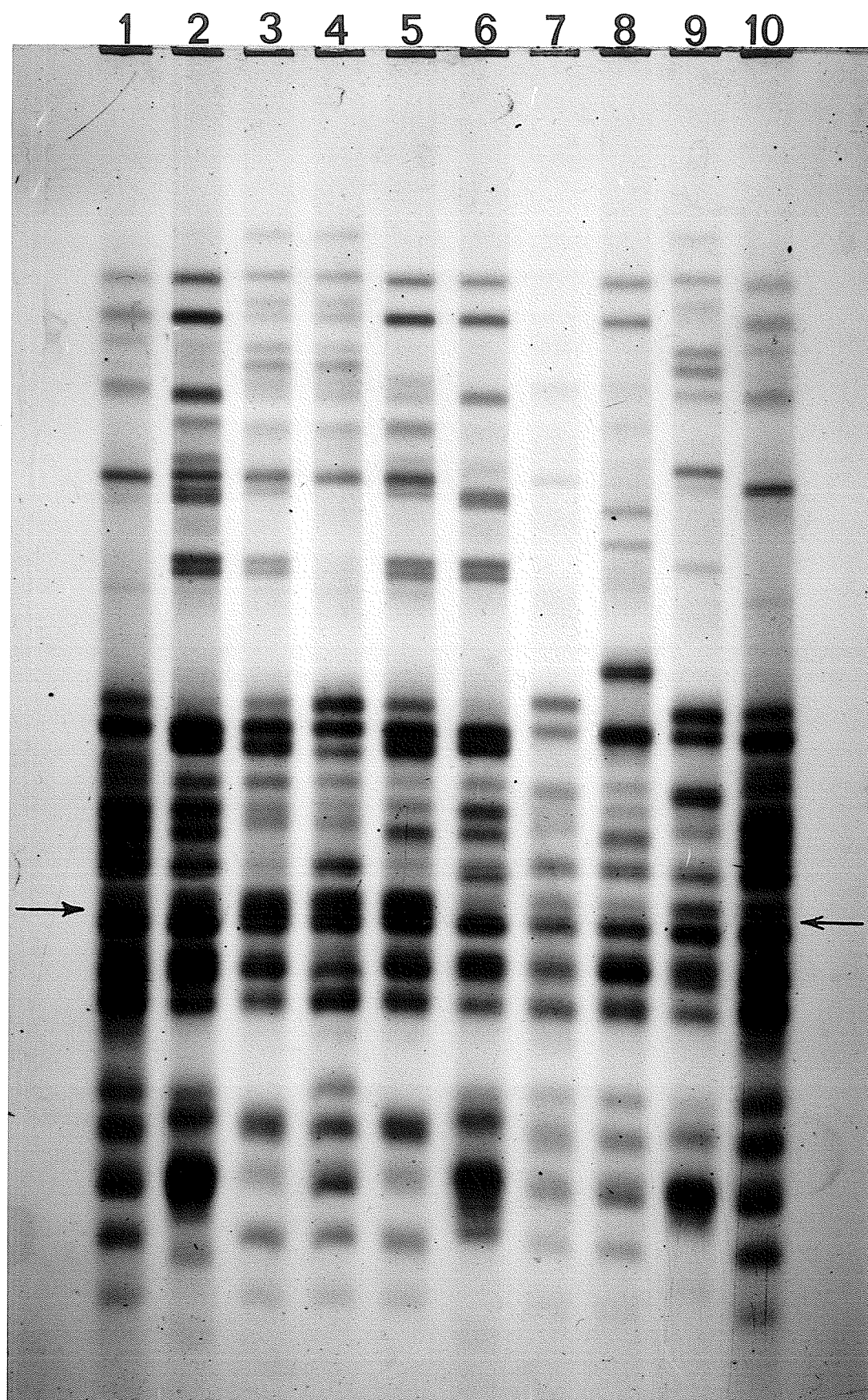
2. Gliadin Electrophoregrams and Baking Potential

The possibility of qualitative and quantitative differences between varieties, of varying baking potential, in the gliadin electrophoregrams was investigated by analysing the electrophoregrams of eight of the Lethbridge samples selected for their high and low loaf volume. These electrophoregrams are shown in Figure 7. Slots numbers 2 to 5 represent high loaf volume varieties, slots numbered 6 to 9 represent low loaf volume varieties, and slots 1 and 10 are again used for the reference variety (Marquis).

While there is marked variability among the eight varieties, there is no obvious characteristic pattern (or part thereof) that can be ascribed to either the high or the low loaf volume varieties. This observation is generally consistent with published information (Elton and Ewart 1964; Doekes 1968; Orth and Bushuk 1972). However there is suggestion that there are quality-related differences in the intermediate (indicated in Figure 7) mobility zone of the electrophoregram. In this zone, all of high loaf volume samples contained the pronounced doublet (indicated by the arrow). Furthermore, all of the varieties (used in this study) that had an acceptable loaf volume had the same doublet (see Appendices I and II). However, there are a few low loaf volume varieties that have the same doublet. Accordingly it can be concluded that while the doublet is a marker of breadmaking quality it is not totally specific. The exception to this generalization

Figure 7. Gliadin electrophoregrams of eight varieties (grown at Lethbridge) selected for high and low remix loaf volume.

	<u>Loaf Volume</u> (cc)
1. Marquis	-
2. Saric 70/Neepawa	1045
3. Kenya 321.BT.1.B.1	1000
4. Chester	1050
5. Sinton	1070
6. H-Ra ² F ₂	570
7. Tesopaco sib	570
8. Sonalika	675
9. Glenlea	620
10. Marquis	-



is the variety Glenlea (slot 9, Figure 7) which gives low loaf volume because of its overly strong dough mixing characteristics (Bushuk 1980). Perhaps the doublet can be used as a marker of strong and very strong dough mixing properties rather than loaf volume as determined by a standard baking test as used in the present study. Further work on more varieties is needed to investigate this possibility.

As an extension of the observation noted above, the gliadin electrophoregrams for the 88 licenced Canadian varieties published by Zillman (1978) were examined for the presence of the characteristic doublet. It was noted that the patterns of the 50 common wheat varieties only those of the hard red spring class deemed equal to Marquis by the licencing process contained the doublet. However, some of other varieties also had the doublet, accordingly it was not possible to use the doublet as a marker of Marquis-type breadmaking quality.

Until recently all of the research in this area had shown that there was no simple relationship between a gliadin electrophoregram and breadmaking quality. However, in 1979 the Soviet workers Sozinov and Popereya (1979) reported that gliadin components are very important and beneficial markers of technological quality. These workers observed that the Sedimentation Values were higher for wheat lines (in a genetic experiment) whose electrophoregram contained gliadin block Gld IA2. Since different electrophoretic methods were used in the Soviet and the present studies, it is not possible to say if the quality related doublet is part of the gliadin block Gld IA2. To interrelate the two

sets of results, it would be necessary to analyze the aneuploid lines used by Sozinov and Popereleya (1979) by the PAGE method used in the present study.

C. Densitometric Analysis of Gliadin Electrophoregrams

In this part of the study, photographic negatives of the electrophoregrams (obtained by PAGE) were scanned on a recording microdensitometer (ORTEC Model 4310 Densitometer) to examine the effect of environment and genotype on densitometric profiles. Two parameters, total gliadin content and gliadin content per unit grain protein, derived from the densitometric profiles, were analyzed to determine if they were related to the remix loaf volume.

1. Effect of Environment on Densitometric Profiles

Four wheat varieties, randomly selected, each grown at the two locations (Lethbridge and Swift Current) were used in this experiment. The gliadins of the eight samples were electrophoresed in a single gel to eliminate potential inter-gel effects. The two electrophoregrams were scanned and the profiles were recorded in superimposed fashion. The duplicate profiles for the four selected varieties are shown in Figures 8 to 11.

For three of the four varieties, the intravariety agreement is considered excellent; the small deviations indicated minor quantitative differences in band intensity. These are considered insignificant,

Figure 8. Densitometric profiles of wheat cv. Neepawa grown at Lethbridge and Swift Current.

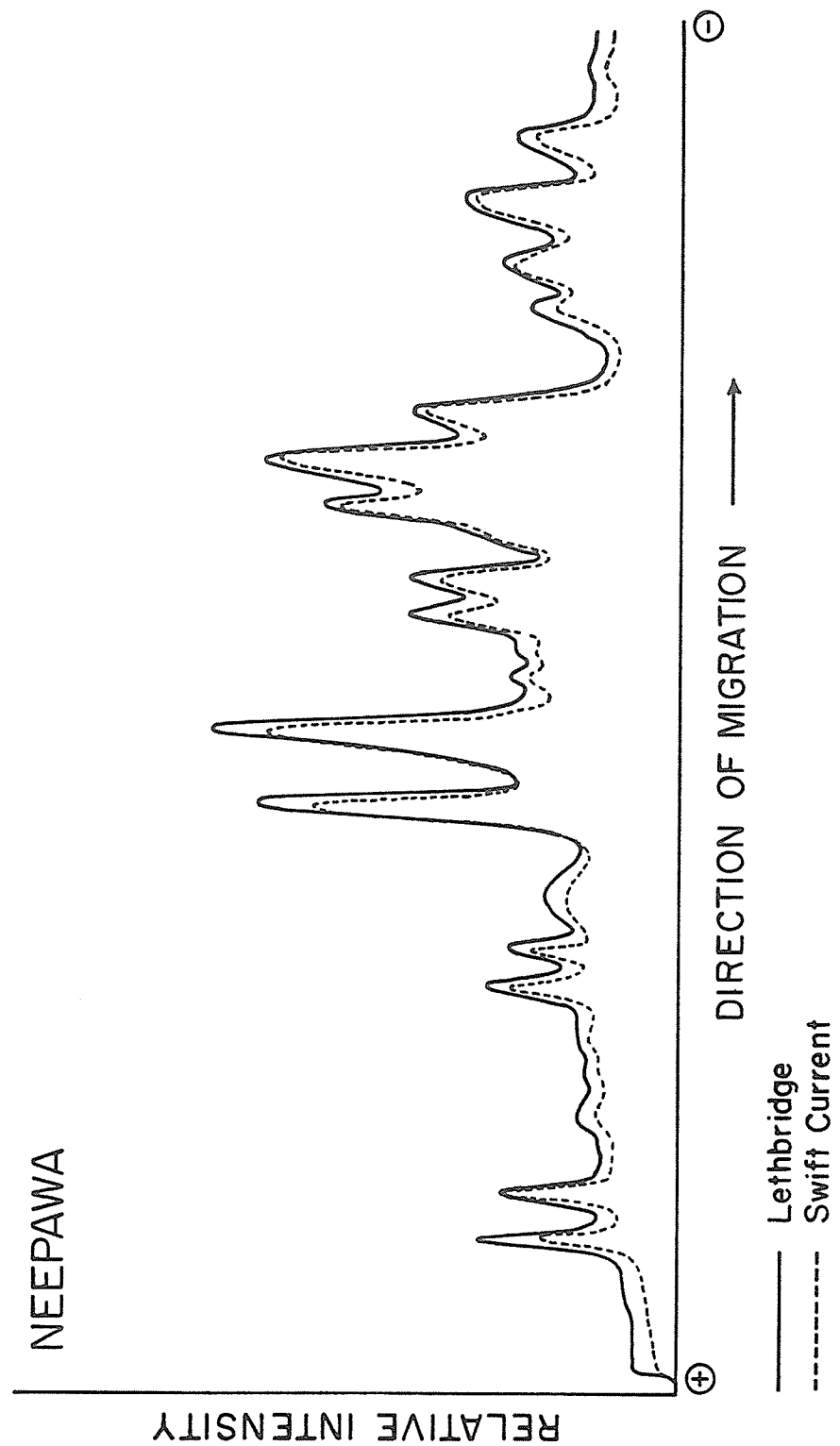


Figure 9. Densitometric profiles of wheat cv.
RL2520//Tc*6/KF grown Lethbridge and
Swift Current.

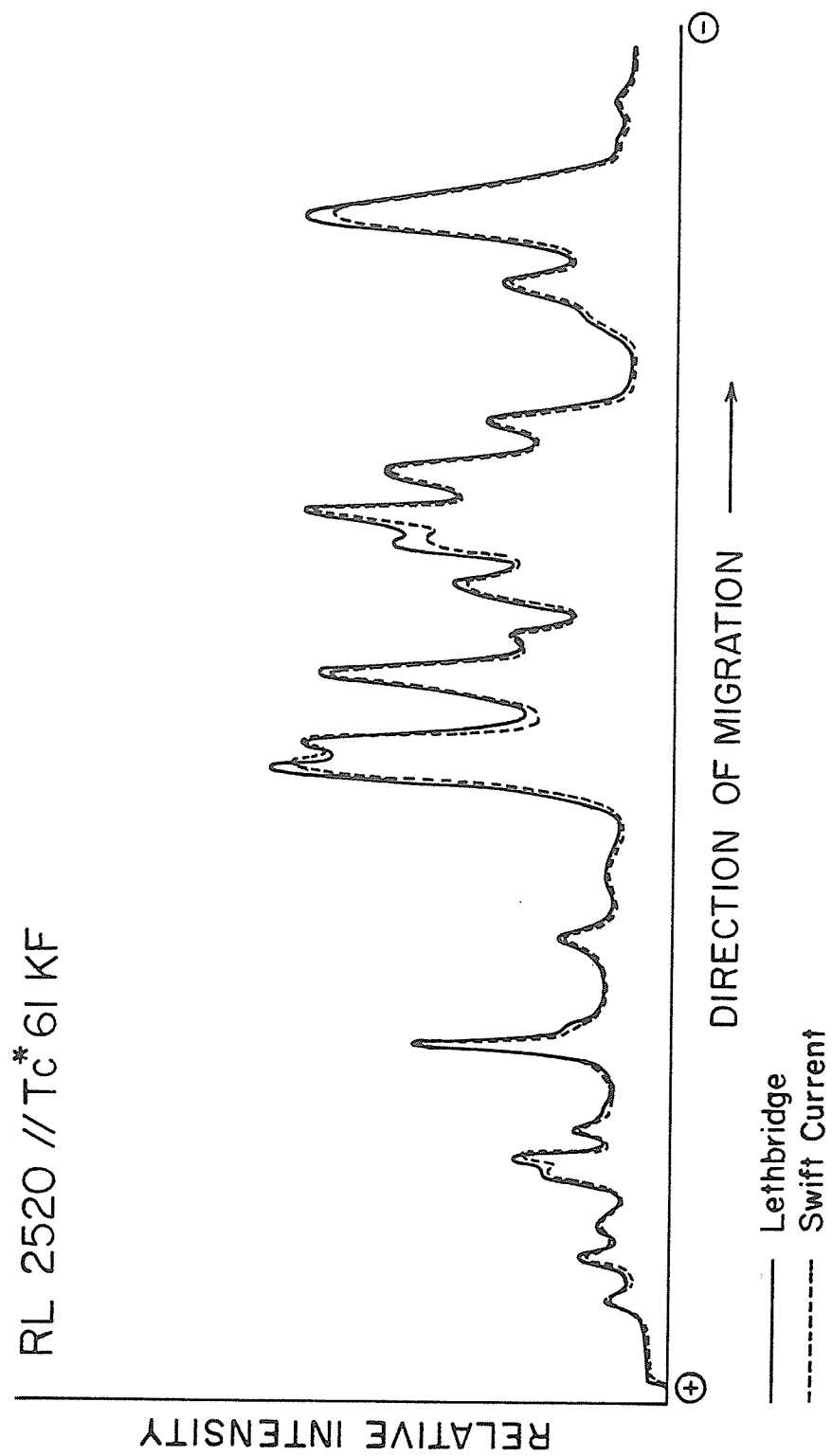


Figure 10. Densitometric profiles of wheat cv. Glenlea grown at Lethbridge and Swift Current.

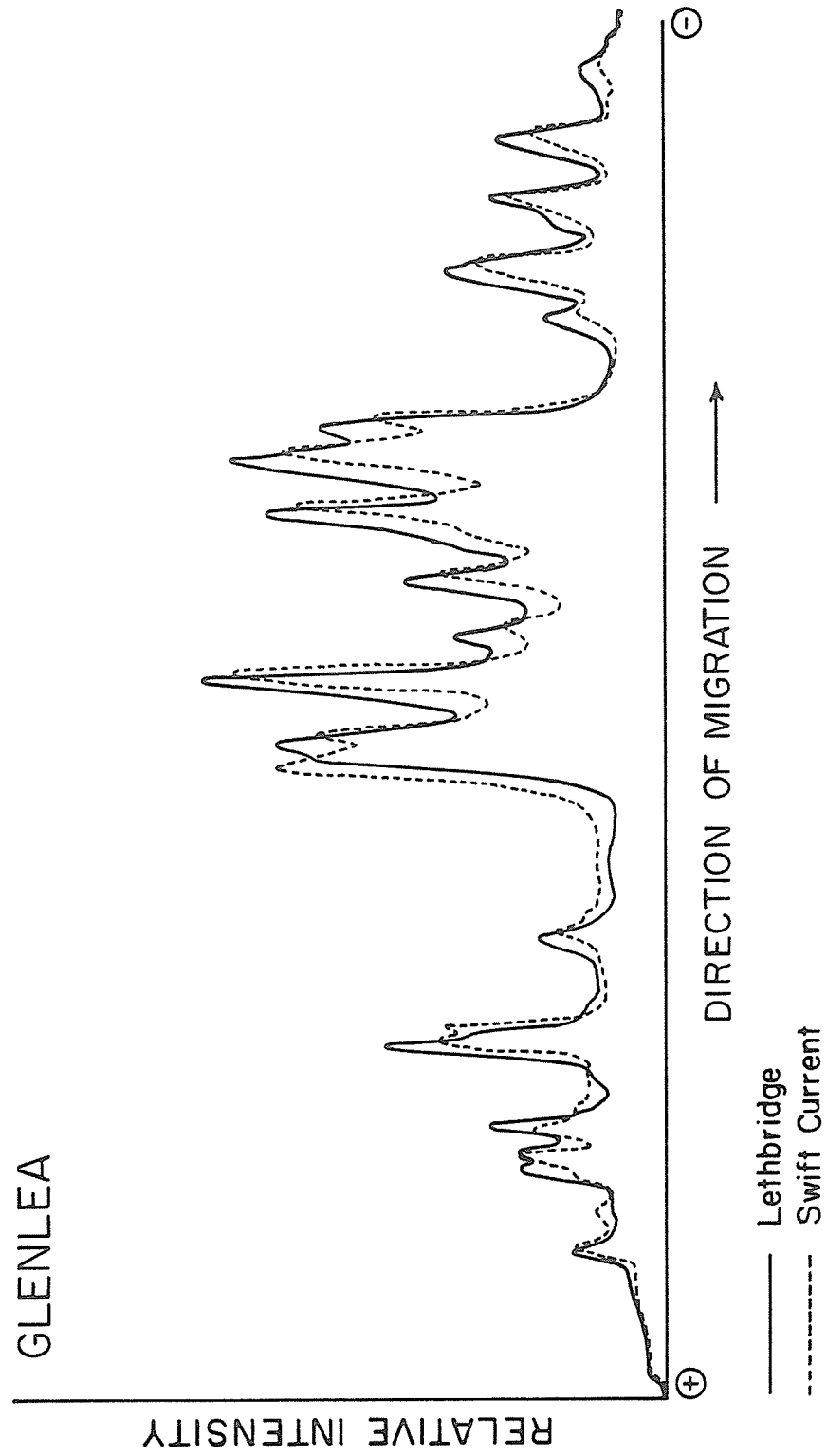
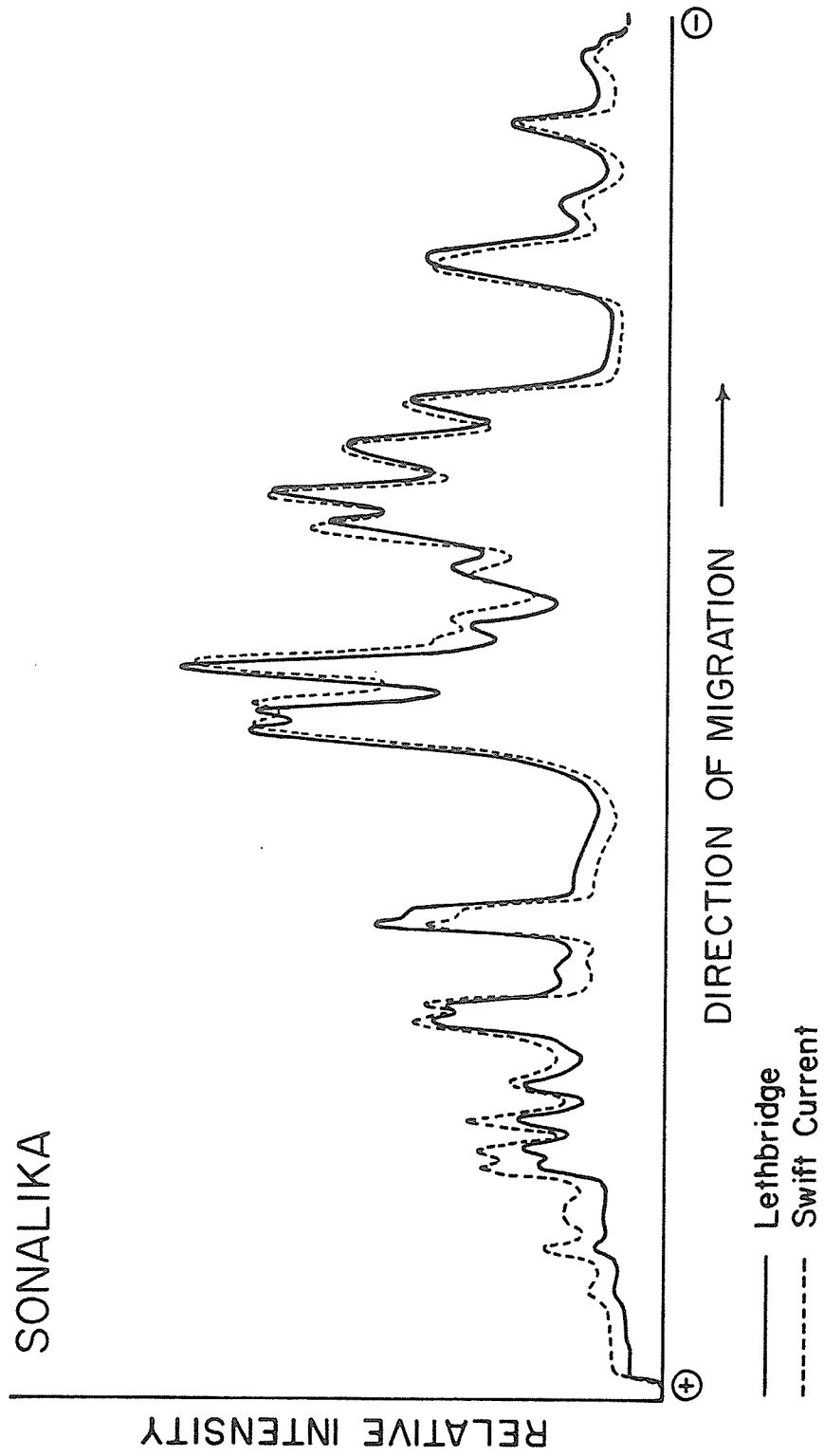


Figure 11. Densitometric profiles of wheat cv. Sonalika grown at Lethbridge and Swift Current.



especially in view of the fact that the densitometric measurements were made from a photographic negative. In some cases, these very small differences were probably related to the difference in protein content between the samples of each variety.

Significant qualitative and quantitative differences were observed between the densitometric profiles for Glenlea (Figure 10). However the differences were not significant to mask the qualitative nature of the Glenlea electrophoregram. The only explanation that can be offered for the inconsistency of the Glenlea results is the known tendency of this variety to outcross which would increase the probability of development of off-types. Further work is needed to apply the electrophoretic technique to the study of the outcrossing tendency of Glenlea and similar varieties.

The results presented here indicate that environment, in general, has little effect on the quantitative aspect of the gliadin electrophoregram.

2. Gliadin Content by Densitometry and Breadmaking Potential

Eight varieties from the Lethbridge samples were selected for this analysis on the basis of their high and low remix loaf volume. In this experiment, the area of the densitometric profile of the gliadin electrophoregram (measured with a planimeter) was taken as an index of the gliadin content. By dividing this value by the protein content of the grain, a value that is directly related to

gliadin content per unit protein was derived.

The data of Table 11 show that the samples with lower loaf volume were characterized by a considerably higher gliadin content. Indeed, for this limited preselected sample, the correlation between loaf volume and gliadin was highly significant ($r = - 0.82^{**}$; Figure 12).

The results presented in this section are quite interesting. They appear to be generally consistent with the conclusion of Hoseney et al. (1969c) who, on the basis of reconstitution experiments with two flours, concluded that the gliadin fraction controlled loaf volume. In the work by Hoseney et al. (1969c), the nature of difference(s) (qualitative and quantitative) between the gliadin fraction from the low- and high-quality flours was not determined.

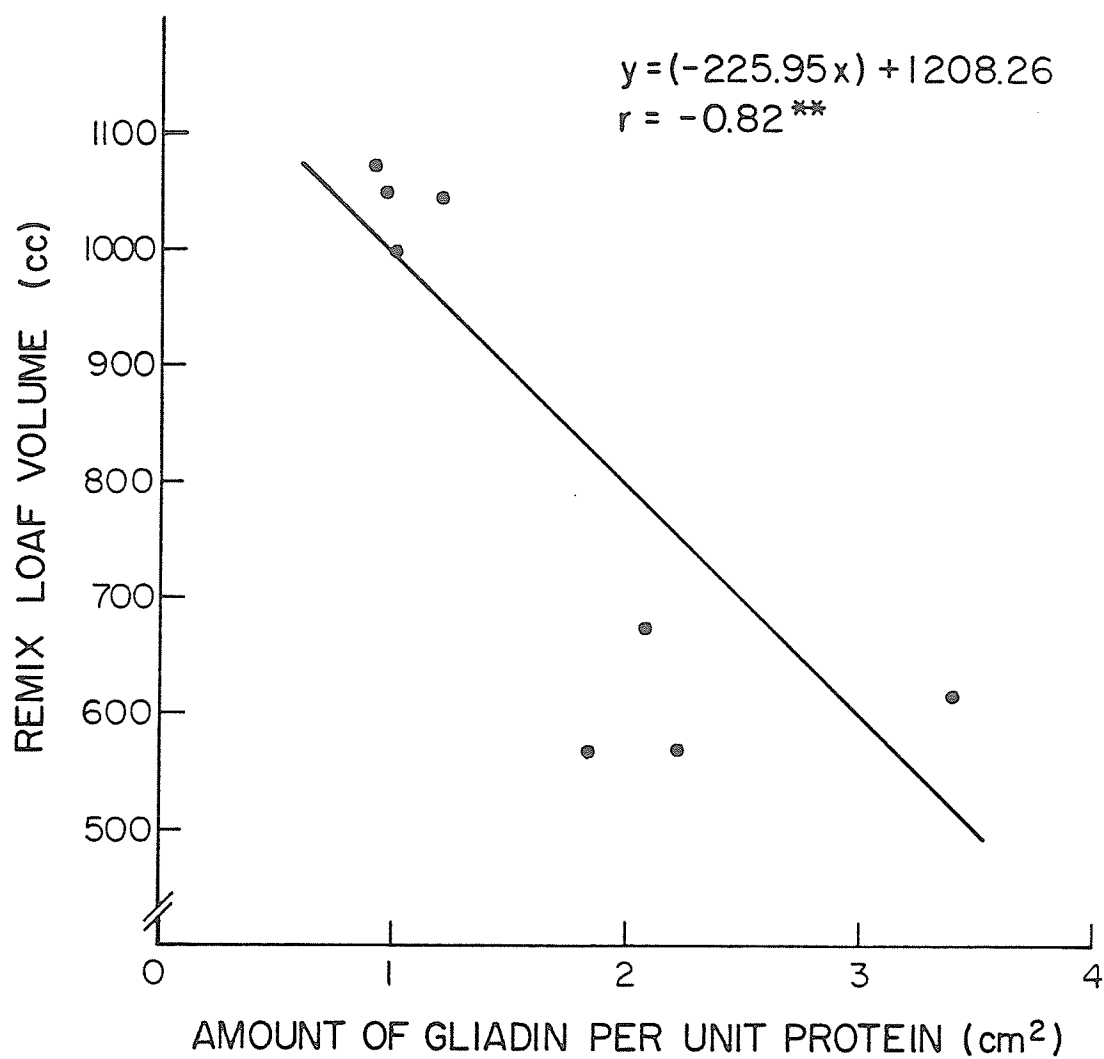
On the other hand, the results appear to disagree with those of Orth and Bushuk (1972). These workers showed that, for a similar set of varieties (but grown at four stations instead of two), the correlation between amount of gliadin, obtained by the modified Osborne fractionation procedure, and loaf volume was not significant at the 5% level. It should be noted that the amount of gliadin, determined by the modified Osborne procedure, may or may not be directly related to the amount determined from the densitometric profile of the gliadin electrophoregram as used in the present study. In PAGE of the 70%

Table 11. Relationship Between Densitometric Gliadin Content
and Loaf Volume

Variety*	Wheat Protein Content (%)	Remix Loaf Volume (cc)	Total Content (cm ²)	Gliadin Per Unit Protein (cm ² /5)
Saric 70/Neepawa	17.2	1045	20.2	1.17
Kenya 321.BT.1.B.1	13.6	1000	13.5	0.99
Chester	14.1	1050	13.6	0.97
Sinton	14.5	1070	13.6	0.94
H-Ra ² F ₂	13.6	570	24.9	1.83
Tesopaco sib	12.0	570	26.5	2.21
Sonalika	12.2	675	25.5	2.09
Glenlea	12.9	620	43.5	3.37

* All grown at Lethbridge

Figure 12. Relationship between loaf volume and densitometric gliadin content per unit protein



ethanol extract of flour, considerable protein remains at the point of extract application (slot). In the modified Osborne procedure, the flour is first extracted with 0.5M sodium chloride solution to remove the albumins and globulins before the gliadin are extracted with 70% ethanol solution.

Further research is needed to clarify the apparent discrepancy between the results obtained in the present study and those of Orth and Bushuk (1972).

D. SDS-PAGE of Glutenin Proteins and Breadmaking Quality

1. Effect of Area of Growth on SDS-PAGE Patterns

The reduced and unreduced glutenin of wheat varieties grown at two locations (Lethbridge and Swift Current) were examined by SDS-PAGE. No qualitative or quantitative differences between the two patterns for each variety were observed.

A very high degree of similarity of the SDS-glutenin patterns was observed among the wheat varieties used in this study. Figure 13 shows the patterns of reduced and unreduced glutenins of five randomly selected varieties grown at the two locations. The SDS-PAGE patterns for the remaining wheat varieties examined are shown in Appendix III. These results show that glutenin patterns are genetically controlled and are not affected by the area of growth. These results are in

Figure 13. SDS-PAGE patterns of glutenin proteins isolated from wheat varieties grown at Lethbridge (L) and Swift Current (SC).

Left side: reduced glutenins

Right side: unreduced glutenins

- | | | | |
|----------------------|------|----------------------|------|
| 1. Era | (SC) | 6. RL 2520//Tc* 6/KF | (L) |
| 2. Era | (L) | 7. Neepawa | (SC) |
| 3. Glenlea | (SC) | 8. Neepawa | (L) |
| 4. Glenlea | (L) | 9. Manitou | (SC) |
| 5. RL 2520//Tc* 6/KF | (SC) | 10. Manitou | (L) |



agreement with published information of Orth and Bushuk (1973b).

2. Glutenin Patterns and Breadmaking Quality

Since the main objective of this study was to examine possible relationships between the electrophoretic properties of wheat gluten proteins and breadmaking quality, the SDS-PAGE electrophoregrams of the reduced and unreduced glutenins of the 26 varieties were analyzed in the context of this objective. This analysis showed that there was no visually obvious relationship between glutenin electrophoregrams and loaf volume. Some varieties that had very different loaf volumes had similar electrophoregrams and vice versa. It should be noted however that, from the results presented here, it is not possible to discern if there are differences in the amounts of equivalent subunits between varieties of different quality. Quantitation of electrophoregram bands (by extraction and analysis) is extremely difficult and lacks accuracy. For these reasons this avenue of investigation was not pursued.

The results obtained in the present study are generally consistent with the previous work of Orth and Bushuk (1973b). However, more recent work of Payne et al. (1979; 1981) showed that there is an apparent relationship between a certain high mol wt glutenin (as observed by SDS-PAGE) and breadmaking quality. These workers showed that this high mol wt subunit was controlled by 1D chromosome. The role of D genome for controlling the inheritance of high mol wt sub-

units in common wheats (as compared with durum wheats which lack the D genome) was established in earlier by Huebner (1970), Bietz and Wall (1972), and Orth and Bushuk (1973c). However, Orth and Bushuk (1973c) reported that the presence or absence of the high mol wt subunits was not sufficient to explain the differences in baking quality among common wheats.

The SDS-PAGE patterns for the unreduced glutenins (Figure 13) are of poor quality because of the excessive streaking. However, examination of the stained gels indicated that this protein contains many low mol wt components that entered the gel. Khan and Bushuk (1979a) reported that these low mol wt proteins, which appear to interact specifically with the high mol wt proteins which do not enter the gel, may contribute to the functional properties of glutenin in breadmaking. The results obtained in the present study indicate that there are no obvious varietal differences in the patterns of unreduced glutenin. Accordingly it is concluded that this fraction of glutenin does not contribute to the varietal differences in breadmaking quality although it may very well contribute to the functionality of glutenin. It would be of interest to study the role of specific glutenin. However the conformational structure of glutenin can not be determined because of its high insolubility. Most researchers used various solvents to dissociate these proteins to facilitate such studies. However, the disruption of the native structure of these proteins may lead to information that is not applicable to aggregated concentrated systems such as doughs. Reconstitution techniques should be used to relate molecular results to functional (rheological) properties of bread dough

which reflect breadmaking quality more directly.

E. Gel-Filtration Chromatography Results

The objective of the experiments described in this section was to examine the possibility that qualitative and quantitative differences may exist between gel-filtration profiles of protein fractions from wheat varieties of different breadmaking quality.

Figure 14 shows the elution profiles of the gliadins (obtained by alcohol extraction -pH precipitation method) of four wheat varieties selected to represent high and low loaf volumes. Two included peaks (I and II) were obtained in each fractionation.

The four profiles were essentially the same. It was noted however the area of peak I decreased while that of peak II increased with decreasing baking quality. This observation could be magnified by determining the ratio of peak I (high mol wt protein) to peak II (low mol wt protein). However the differences observed are considered too small to be a reliable index of baking quality for screening populations in breeding programs.

Figure 15 shows the elution profiles of the glutenin fraction of the same four samples used for the gliadin experiment described above.

As in the case of the gliadin profiles (Figure 14), two peaks (I and II) were obtained for each sample. For all samples examined,

Figure 14. Gel-filtration elution profiles on Sephadex G-200 of the gliadins of wheat varieties of different baking potential (Lethbridge Samples). The column size was 2.5 x 100 cm and the flow rate was 9 ml/hr. Fraction volume was 4.5 ml/tube.

	<u>Loaf Volume</u> (cc)
1. Sinton	1070
2. Chester	1050
3. Tesopaco sib	570
4. H-Ra ² F ₂	570

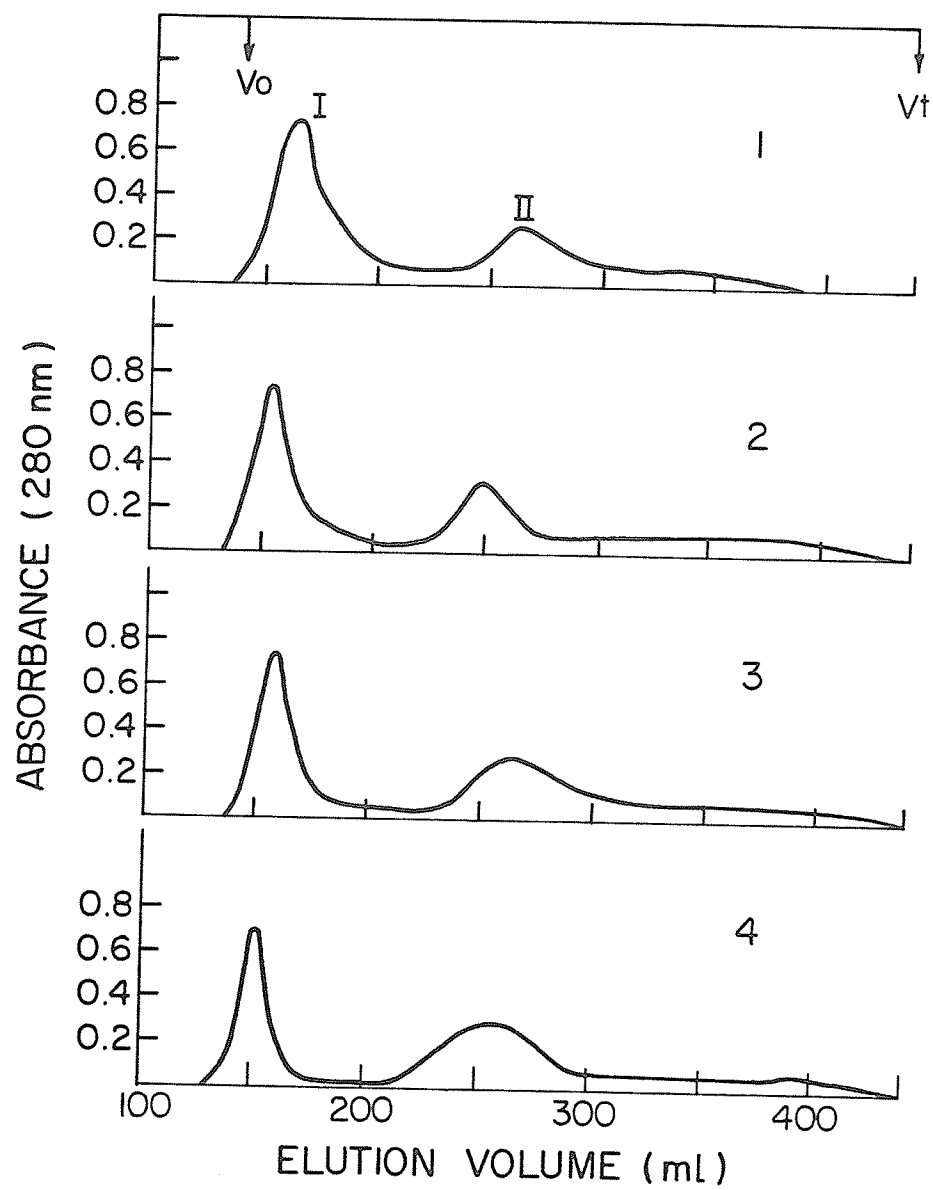
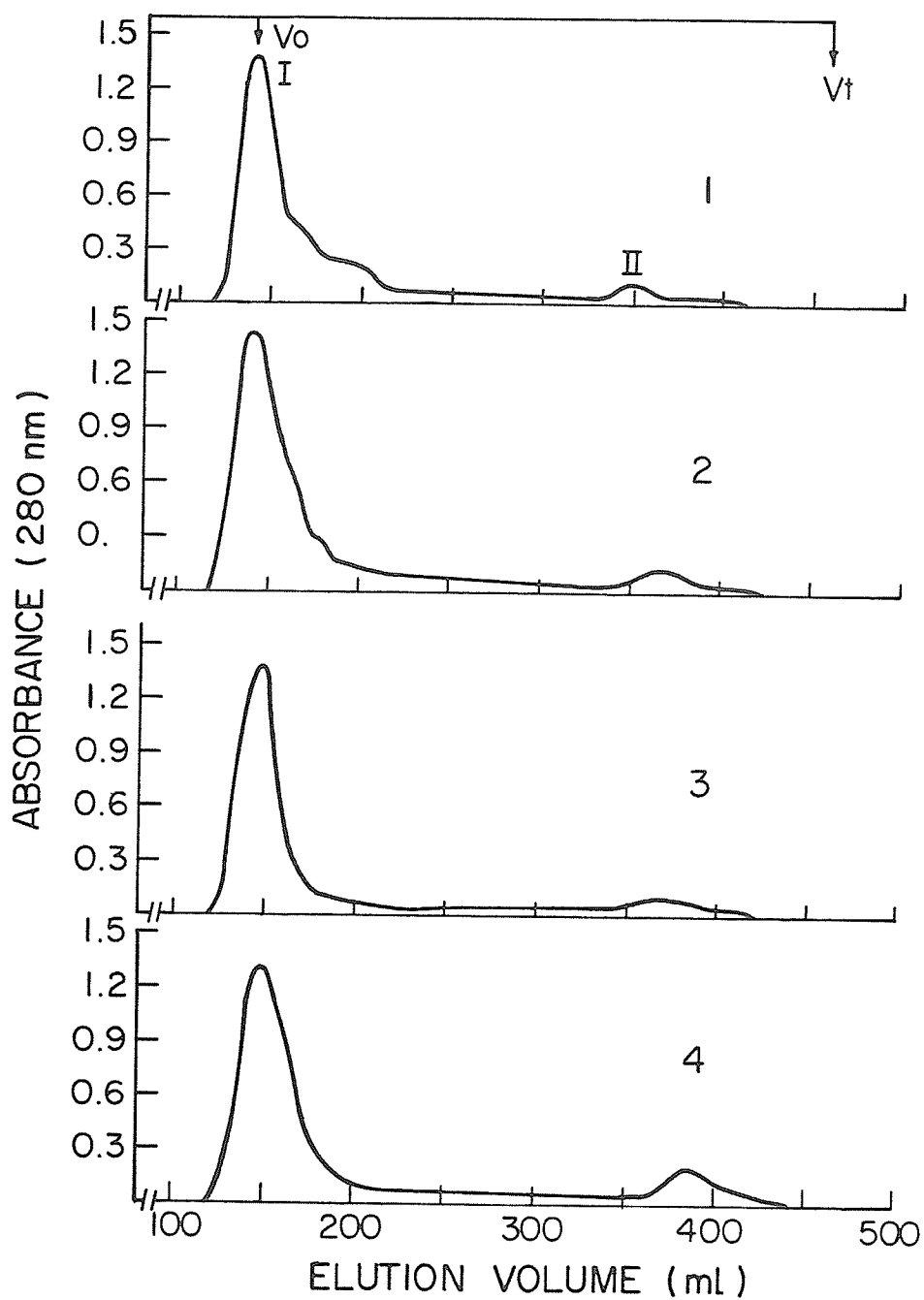


Figure 15. Gel-filtration elution profiles on Sephadex G-200 of glutenins of wheat varieties of different baking potential (Lethbridge samples). The column size was 2.5 x 100 cm and the flow rate was 9 ml/hr. Fraction volume was 4.5 ml/tube.

	<u>Loaf Volume</u> (cc)
1. Sinton	1070
2. Chester	1050
3. Tesopaco sib	570
4. H-Ra 2F_2	570



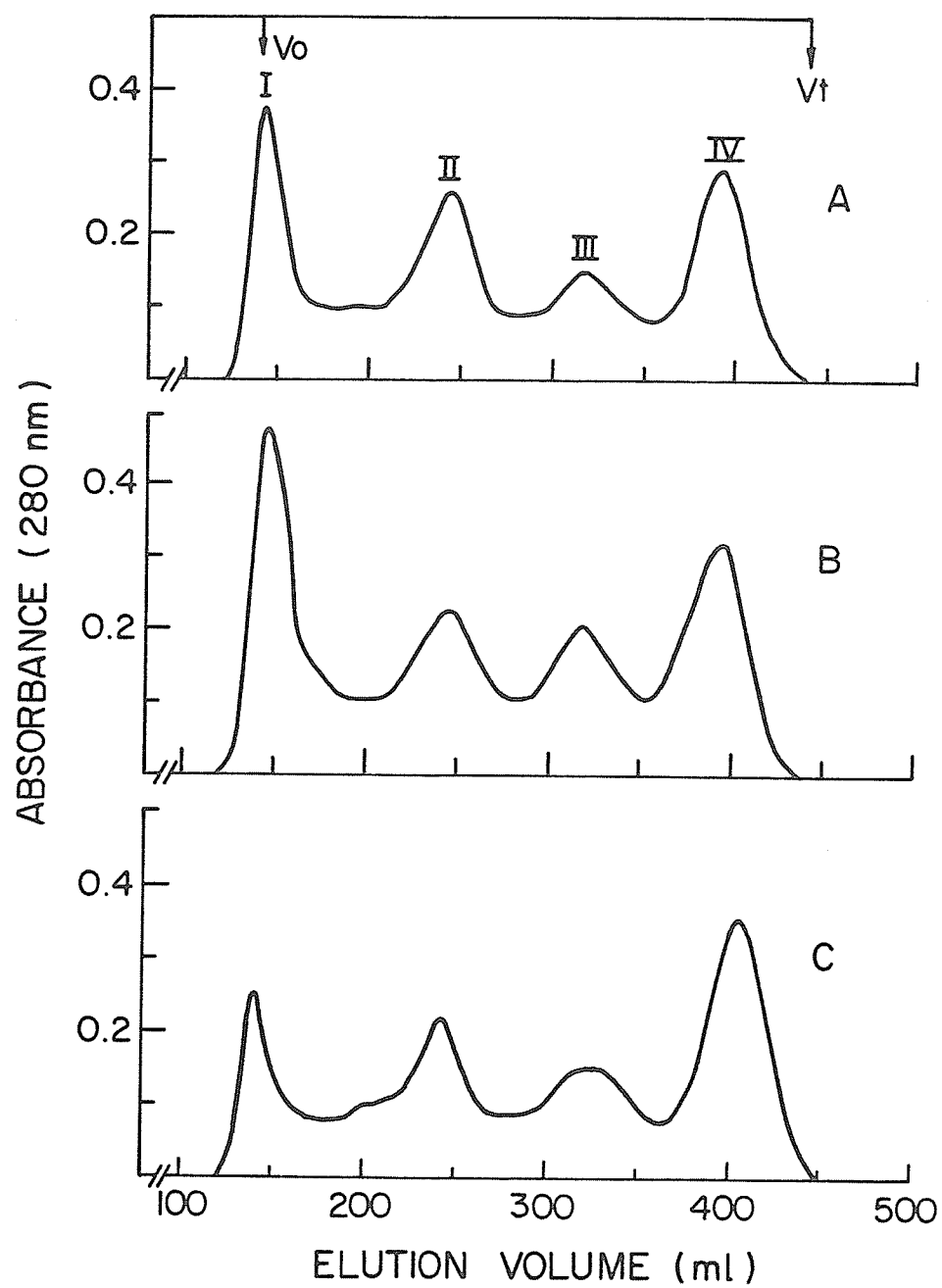
peak I was eluted in the void volume, indicating an apparent mol wt higher than 200,000. Two minor differences were observed for peak I between the two high loaf volume and the two low loaf volume samples. Firstly, peak I for the samples of low loaf volume eluted at a higher elution volume (indicating a lower mol wt). Secondly, this peak for the higher loaf volume samples had slower-eluting shoulder which was not present in the profiles of the low quality samples. More work is required to investigate the nature of this shoulder in the elution profiles of the high loaf volume sample.

On other hand, peak II showed a high degree of similarity among the four samples investigated. Accordingly a further gel-filtration experiment was carried out on the total protein extracted with the dissociating solvent AU (0.1N acetic acid and 3M urea). In this experiment, three samples were selected on the basis of dough development time (mixing strength) and loaf volume. The results for these samples are shown in Figure 16. Sample 'A' (Sinton) represents a strong variety with a long dough development time and high loaf volume. Sample 'B' (Sonalika) represents a weak variety with a short dough development time and low loaf volume. Sample 'C' (Glenlea) represents an overly strong variety which gives a low loaf volume because its gluten is not adequately developed by the constant mixing time of the remix baking test.

The profiles obtained in this experiment were characterized by four peaks. These peaks are generally referred to as glutenin (peak I),

Figure 16. Gel-filtration elution profiles on Sephadex G-200 of Au extracts of wheat varieties of different dough strength and baking potential (Lethbridge Samples). The column size was 2.5 x 100 cm and the flow rate was 9 ml/hr. Fraction volume was 4.5 ml/tube.

	<u>Loaf Volume</u>
	(cc)
A. Sinton	1070
B. Sonalika	625
C. Glenlea	620



gliadin (peak II), water-soluble proteins (peak III) and non-protein molecules (peak IV) (Meredith and Wren 1966).

The latter three peaks (II, III and IV) were similar for all three samples; that is there were no differences which could be attributed to differences in dough strength or baking potential. On the other hand, the area of peak I was similar for the two weaker but considerably higher than the area for the overly strong variety (Glenlea). These results are generally consistent with the solubility fractionation results of Orth and Bushuk (1972). While the results of this study suggest a relationship between the high mol wt glutenin and dough mixing properties, the difference between the two strong varieties (Glenlea and Sinton) is not sufficiently large to make this parameter suitable for quality screening purposes.

VI. GENERAL DISCUSSION

Protein is the component of wheat flour that accounts for the major portion of its breadmaking quality. In this function, both the quality and the quantity of the protein are important. The quantity of protein depends primarily on the conditions during the growing season, especially on the amount of nitrogen in the soil. The quality of the protein is primarily an inherited characteristic which can be affected by the growing conditions. Wheat varieties (cultivars if they are grown commercially) can be distinguished by their protein quality. Many of the properties of flour proteins that contribute to breadmaking quality are known. However our knowledge on this subject is far from complete. Some new information has been added to the pool of knowledge already accumulated from the results of this thesis project.

This study was undertaken to examine primarily the electrophoretic properties of gluten proteins of 26 varieties of breadwheat of different baking quality grown at two locations. The study was extended to include other experiments such as solubility fractionation and gel-filtration. The objective was to identify or confirm the factor(s) responsible for the intervarietal differences in quality.

As found by many previous investigators, large and obvious differences were observed between PAGE electrophoregrams of the gliadins of different varieties. The location of growth had no effect

on the qualitative nature of the electrophoregram. Differences in the intensity of some bands observed for some varieties can be explained on the basis of differences in protein content of the grain samples of the same variety.

Electrophoregrams of the gliadin of eight varieties of widely different baking potential were examined in detail. This analysis showed definite differences between the electrophoregrams of the high and low loaf volume varieties. The presence of a readily-identified doublet was characteristic of the high loaf volume samples. However when the electrophoregrams for all 26 varieties were examined, several exceptions to this observation were noted. Accordingly, it cannot be generalized that the presence of this doublet is required for high baking potential. However, the doublet can be used as a marker of baking quality in the early generation screening to eliminate low quality varieties. Other tests must be applied to eliminate the low quality varieties that have the doublet. More work is required on the genetics and baking quality implications of this widely spread doublet. Also it would be of special interest to determine if the doublet identified in the present study is part of the allelic gliadin block identified by Sozinov and Popereya (1979) as the requirement for high technological quality (high sedimentation value).

The independency of gliadin electrophoregram of the area of growth was confirmed, in this project, by examining the electrophoregrams of four randomly selected cultivars.

The present study showed that the proportion of gliadin in flour protein appears to be related to differences in baking potential between varieties. Results showed that the gliadin content (as determined from densitometric profiles of gliadin electrophoregrams) was highly significantly correlated with loaf volume. The correlation was negative indicating that a higher proportion of gliadin leads to a lower baking potential. These results are generally consistent with the finding of Hoseney et al. (1969c) but appear contrary to the observation of Orth and Bushuk (1972) who observed that the amount of gliadin obtained by the modified Osborne fractionation was not significantly correlated with loaf volume. Further work is needed to establish the relationship between the gliadin content determined by the densitometric method (used here) and the Osborne fractionation technique before any further comment can be made on the apparent inconsistency.

Reduced and unreduced glutenins of the 26 varieties, grown at two locations, were examined by SDS-PAGE. No obvious qualitative or quantitative differences in the patterns of reduced glutenins were observed that can be related to differences in baking quality. As in the case of the gliadin electrophoregrams, the glutenin electrophoregrams were also independent of growth conditions. These results are in general agreement with published information in this area (Orth and Bushuk 1972; Butaki and Dronzek 1979). However, the work of Payne et al. (1979, 1981) on glutenin subunits showed that a high mol wt subunit (145,000) controlled by 1D chromosome is generally required for baking quality.

The results of the present study are not of sufficient clarity to either confirm or contradict the results of Payne et al. (1979, 1981). A special extended electrophoresis - time technique and gel concentration were used by these authors.

The SDS-PAGE electrophoregrams of the unreduced glutenins are characterized by two groups of protein subunits (Khan and Bushuk 1979a). One group of low mol wt enters the gel and the other of high mol wt and remains at sample application slot. While the electrophoregrams of the unreduced glutenins were not very clear, examination of the stained gels indicated no obvious differences between varieties of different baking quality.

Gel filtration profiles on Sephadex-G200, of gliadin and glutenin fractions extracted from four wheat varieties of different baking potential, did not reveal differences among the samples investigated that can be related to differences in baking potential. However, there were quantitative differences between gel filtration profiles of the total AU extracts of three wheat varieties which varied in dough strength and baking potential. The overly strong variety (Glenlea) had the lowest amount of glutenin (peak I) in comparison with the other wheat varieties used in this study. This result is generally consistent with the Osborne fractionation results of Orth and Bushuk (1972) who showed that the amount of glutenin was negatively related to loaf volume. It should be emphasized, however, that the amount of glutenin by gel-filtration of AU extracts (peak I) may not be equivalent to the

amount of the glutenin fraction of the Osborne fractionation. Further work is needed to interrelate the results of the two methods.

VII. SUMMARY

1. Statistical analyses of the technological data for the 26 wheat varieties of the 1979 Uniform Quality Nursery, grown at Lethbridge and Swift Current, gave additional information on the relationship between wheat protein (quality and quantity) and loaf volume potential.
2. Farinograph water absorption and protein content were found to be the best indicators of loaf volume potential. On the other hand, Zeleny sedimentation value and dough development time were not highly significantly correlated with loaf volume.
3. The intervarietal and interstation effects on the intrinsic quality of the flour protein (as measured by loaf volume per unit protein and BSI Test) showed a great variation in protein quality among varieties and between the samples of the same variety grown at the two locations.
4. Improved resolution of gliadin components by PAGE was obtained using sodium lactate buffer instead of the classical aluminum lactate.
5. As found by other workers, gliadin electrophoregrams were independent of area of growth and marked differences were observed among the varieties.
6. A characteristic doublet was present in the gliadin electrophoregrams of all varieties of high baking quality. Varieties that did not have this doublet were of low quality. Some low quality varieties also

contained the doublet.

7. Gliadin content (as determined from the densitometric profiles of gliadin electrophoregrams) was positively correlated with loaf volume potential.

8. Urea can be completely removed from AUC extracts of flour by using magnetic stirring during dialysis. This is important when the Kjeldahl method is used to analyze AUC extracts for nitrogen.

9. No relationship could be established between glutenin (reduced and unreduced) SDS-PAGE electrophoregrams and breadmaking quality.

10. As found by others, this study showed that glutenin electrophoretic patterns were independent of location of growth and seemed to be genetically controlled.

11. Gel-filtration profiles of wheat protein fractions (gliadin and glutenin) did not show differences that can be related to differences in loaf volume potential. Quantitative differences were evident in the profiles of AU extracts among wheat varieties of different dough strength and baking potential.

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APPENDIX I
Quality Data for 26 varieties
Grown at Lethbridge (L) and
Swift Current (SC)

WHEAT QUALITY TEST PARAMETERS

Test Parameter		Units	Method
Hwt	Hectoliter weight	Kg/hl	#1 below
MKwt	Thousand kernel weight	g	#2 below
Wpro	Grain protein content (N x 5.7; 14% m.b.)	%	AACC Method 46-12
FN	Hagberg falling number	sec	AACC Method 56-81B
PR	Pearling resistance	g	#3 below
Yld	Flour yield (14% m.b.)	%	AACC Method 26-20
FPro	Flour protein content (N x 5.7; 14% m.b.)	%	AACC Method 46-12
Ash	Flour ash content (14% m.b.)	%	AACC Method 08-01
Sed	Zeleny Sedimentation value	cc	AACC Method 56-60
Amyl	Amylograph viscosity	B.U.	AACC Method 22-10
FAbs	Farinograph absorption	%	AACC Method 54-21
DDT	Dough development time	min	AACC Method 54-21
MTI	Mixing tolerance index	B.U.	AACC Method 54-21
RAbs	Baking absorption	%	Usually 4% < FAbs
RLV	Remix loaf volume	cc	#4 below
BLV	Blend loaf volume	cc	#5 below

1. Pour grain into half-liter measure, strike-off and weigh. Multiply weight by 200.
2. Count out 100 mature, sound kernels and weigh. Multiply by 10.
3. Pearl 20 g of grain (13% m.b.) for 20 sec. in a Strong-Scott barley pearler. Record weight of pearls.
4. The G.R.L. MPB (Malt-phosphate-bromate) Remix Pup Loaf Bake Test is used.
5. The sample being tested is blended with an equal weight of soft white wheat flour.

1979 UNIFORM QUALITY NURSERY TEST

Variety or Cross	GRAIN							FLOUR			FARINOGRAPH			BAKING			
	Hwt	MKwt	wPro	FN	PR	% Bran	Yld	FPro	Ash	Sed	Amy1	FAbs	DDT	MTI	RAbs	RLV	BLV
Manitou SC L	77.6	28.4	14.4	445	12.4	18.5	74.1	13.8	0.40	72	800	64.5	6.5	25	60.5	915	705
	75.9	27.6	13.8	440	13.2	19.6	72.4	13.1	0.36	70	860	61.5	5.5	25	57.5	980	760
Neepawa SC L	78.8	30.2	14.1	465	12.6	19.0	73.1	13.7	0.38	71	800	65.4	6.0	25	61.4	925	750
	76.0	27.4	13.9	387	13.0	20.0	71.2	13.1	0.34	71	840	62.9	5.0	30	58.9	970	770
RL2520//Tc*6/KF SC L	79.4	30.9	13.9	495	13.1	17.4	74.3	13.3	0.39	71	1140	66.0	6.0	30	62.0	930	770
	77.5	29.2	13.8	448	14.1	19.4	72.7	13.0	0.36	71	1200	64.3	6.5	25	60.3	975	760
Glenlea SC L	77.8	39.7	13.9	380	12.9	16.9	72.9	13.2	0.42	63	530	63.4	3.0	5	59.4	585	820
	75.1	37.8	12.9	308	14.0	17.4	71.9	12.0	0.39	67	640	62.1	4.0	5	58.1	620	840
Era SC L	79.2	30.5	13.3	430	12.7	16.9	74.3	12.3	0.47	64	560	62.0	6.5	35	58.0	835	805
	78.8	29.6	12.0	388	14.5	17.5	72.3	11.3	0.41	60	770	61.0	5.5	35	57.0	915	655
Sonalika SC L	77.2	42.3	12.5	450	11.3	19.8	72.3	11.5	0.41	51	740	62.2	3.0	65	58.2	710	615
	76.8	42.2	12.2	427	11.5	19.5	72.2	11.1	0.38	40	790	60.6	2.5	65	56.6	675	535
Sinton SC L	80.6	32.9	14.5	450	12.4	19.3	73.1	13.6	0.40	73	820	64.9	8.0	10	60.9	890	750
	77.7	30.8	14.5	446	13.6	20.7	71.1	13.6	0.35	72	840	64.6	7.0	20	60.6	1070	650

1979 Uniform Quality Nursery Test - Continued

Variety or Cross	GRAIN						FLOUR			FARINOGRAPH			BAKING				
	Hwt	MKwt	WPro	FN	PR	% Bran	Yld	FPro	Ash	Sed	Amy1	RAbs	DDT	MTI	RAbs	RLV	BLV
Chester SC L	78.7	32.7	14.6	365	13.1	18.0	73.6	14.0	0.38	74	820	64.8	6.5	10	60.8	945	805
	76.3	29.9	14.1	291	13.7	18.9	72.1	13.3	0.34	73	660	64.4	7.5	25	60.4	1050	735
Kenya 321.BT.1.B.1 SC L	78.0	29.2	13.8	380	12.8	18.4	73.9	13.3	0.41	73	770	61.6	7.5	20	57.6	995	755
	76.7	29.9	13.6	318	13.4	19.4	72.3	12.6	0.37	73	630	61.3	5.0	25	57.3	1000	680
Mida/Cadet SC L	77.1	34.6	14.6	465	12.3	19.5	73.0	14.0	0.43	73	1020	62.9	6.0	10	58.9	1030	705
	78.8	36.7	14.1	400	13.9	19.5	72.1	13.1	0.38	73	880	61.9	6.5	35	57.9	990	610
Saric 70/Neepawa SC L	78.4	32.3	16.7	361	11.6	18.7	72.6	15.9	0.40	72	610	66.4	5.5	35	62.4	1060	650
	77.3	34.5	17.2	315	11.9	19.7	70.9	16.5	0.36	73	400	65.8	4.5	30	61.8	1045	635
Pavon sib SC L	79.6	32.8	13.8	469	13.1	18.5	71.5	13.0	0.41	73	890	63.9	7.0	30	59.9	1010	780
	76.0	32.1	13.4	354	13.6	17.7	71.5	12.5	0.37	71	670	63.5	5.0	55	59.5	875	645
Tesopaco sib SC L	76.2	37.1	13.1	416	9.4	24.2	63.1	12.2	0.42	73	1140	56.2	5.0	60	52.2	490	450
	76.2	35.1	12.0	360	10.4	23.4	62.2	11.2	0.37	67	1080	55.1	3.5	70	51.1	570	525
JIT-35-2L SC L	78.0	34.9	15.0	487	13.2	20.6	71.8	14.4	0.43	73	700	64.1	9.5	0	60.1	890	700
	77.7	36.0	15.4	437	13.1	19.2	72.0	14.5	0.36	73	720	63.5	9.0	25	59.5	995	

1979 Uniform Quality Nursery Test - Continued

Variety or Cross	GRAIN					FLOUR			FARINOGRAPH			BAKING					
	Hwt	MKwt	WPro	FN	PR	% Bran	Yld	FPro	Ash	Sed	Amy1	FAbs	DDT	MTI	RAbs	RLV	BLV
H-Ra 2F2 SC L	75.7	37.2	14.0	375	7.1	25.6	63.6	12.9	0.38	72	920	53.9	5.5	15	49.9	440	420
	75.1	37.6	13.6	344	10.0	25.8	60.7	12.5	0.33	71	900	54.4	5.5	30	50.4	570	520
Bulbu1 SC L	78.3	33.3	13.1	454	13.1	19.1	71.4	12.3	0.39	66	1080	66.5	5.0	45	62.5	875	755
	80.4	34.9	12.1	414	14.3	19.1	70.4	11.0	0.35	48	1120	64.3	4.0	40	60.3	705	420
M.J. INTA SC L	80.7	41.4	14.9	481	12.9	18.3	73.4	13.7	0.37	70	730	64.6	10.0	5	60.6	750	815
	76.2	38.9	14.4	415	13.6	19.0	71.4	12.9	0.33	70	760	62.2	9.0	20	58.2	850	605
ND 560 SC L	80.3	34.7	14.5	500	12.2	17.6	74.6	13.7	0.34	72	860	65.9	7.5	20	61.9	950	805
	78.9	31.6	14.4	453	14.2	19.3	72.1	13.5	0.31	73	880	62.2	8.0	25	58.2	850	580
ND 563 SC L	79.4	35.2	14.9	439	12.9	20.8	70.1	14.4	0.36	74	980	65.2	7.0	10	61.2	805	815
	78.3	33.1	14.3	400	13.5	21.4	68.1	13.6	0.34	73	920	62.3	9.5	15	58.3	855	615
James (SD 2273) SC L	78.1	33.7	14.0	510	11.1	16.8	76.6	13.6	0.39	72	780	63.3	7.0	35	59.3	945	690
	78.2	31.9	14.4	378	11.3	18.1	75.2	13.6	0.34	69	740	60.8	7.5	40	56.8	705	510
SD 2235 SC L	77.8	34.0	14.3	433	12.2	17.6	74.2	13.7	0.40	73	830	64.4	7.0	30	60.4	1005	785
	76.2	32.7	14.8	436	13.9	19.8	71.2	13.7	0.36	69	920	64.0	5.0	30	60.0	875	550

1979 Uniform Quality Nursery Test - Continued

Variety or Cross	GRAIN					FLOUR			FARINOGRAPH			BAKING					
	Hwt	MKWt	WPro	FN	PR	% Bran	Yld	FPro	Ash	Sed	Amy1	FAbs	DDT	MTI	RAbs	RLV	BLV
Len (ND 543) SC L	79.0	34.8	14.2	456	12.6	16.7	75.4	13.6	0.42	71	800	64.6	13.0	15	60.6	895	850
	77.8	31.2	14.4	378	13.6	19.4	71.8	13.3	0.35	71	880	63.0	7.5	20	59.0	900	500
Mn 70170 SC L	78.7	31.9	13.4	539	12.0	17.6	75.0	12.8	0.40	66	920	60.5	5.5	30	56.5	925	710
	78.2	28.9	12.9	388	14.0	17.2	74.1	12.1	0.36	56	960	57.8	5.5	30	53.8	820	585
NAPB NHS 183.74 SC L	76.6	33.9	13.2	350	12.1	19.4	73.8	12.8	0.39	73	940	57.8	7.0	40	53.8	945	705
	77.1	35.0	12.5	384	12.8	18.9	73.7	11.8	0.36	70	920	56.5	6.0	35	52.5	825	550
NAPB NHS 1001-75 SC L	79.7	31.9	13.0	480	12.6	15.9	75.0	12.2	0.43	66	830	60.5	6.5	40	56.5	885	750
	78.8	30.0	12.0	371	14.0	17.3	72.1	11.0	0.38	55	740	59.5	6.0	25	55.5	795	585
CT 790 SC L	79.6	31.0	14.7	488	12.6	18.4	73.0	14.3	0.36	74	940	64.0	8.0	15	60.0	915	675
	75.4	28.0	14.4	379	13.2	19.6	71.3	13.5	0.35	74	840	61.5	8.0	20	57.5	890	575

APPENDIX II

Gliadin Electrophoregrams of
Wheat Varieties Grown at Lethbridge
And Swift Current

Figure 17. Gliadin electrophoregrams of wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Figure in parentheses is the protein content
(N x 5.7, 14% m.b.)

- | | | |
|-------------------------------|--|------------------------------|
| 1. Marquis | | 6. Mida/Cadet (L)
(14.1%) |
| 2. - | | |
| 3. Pavon sib (SC)
(13.8%) | | 7. Chester (SC)
(14.6%) |
| 4. Pavon sib (L)
(13.4%) | | 8. Chester (L)
(14.1%) |
| 5. Mida/Cadet (SC)
(14.6%) | | 9. - |
| | | 10. Marquis |

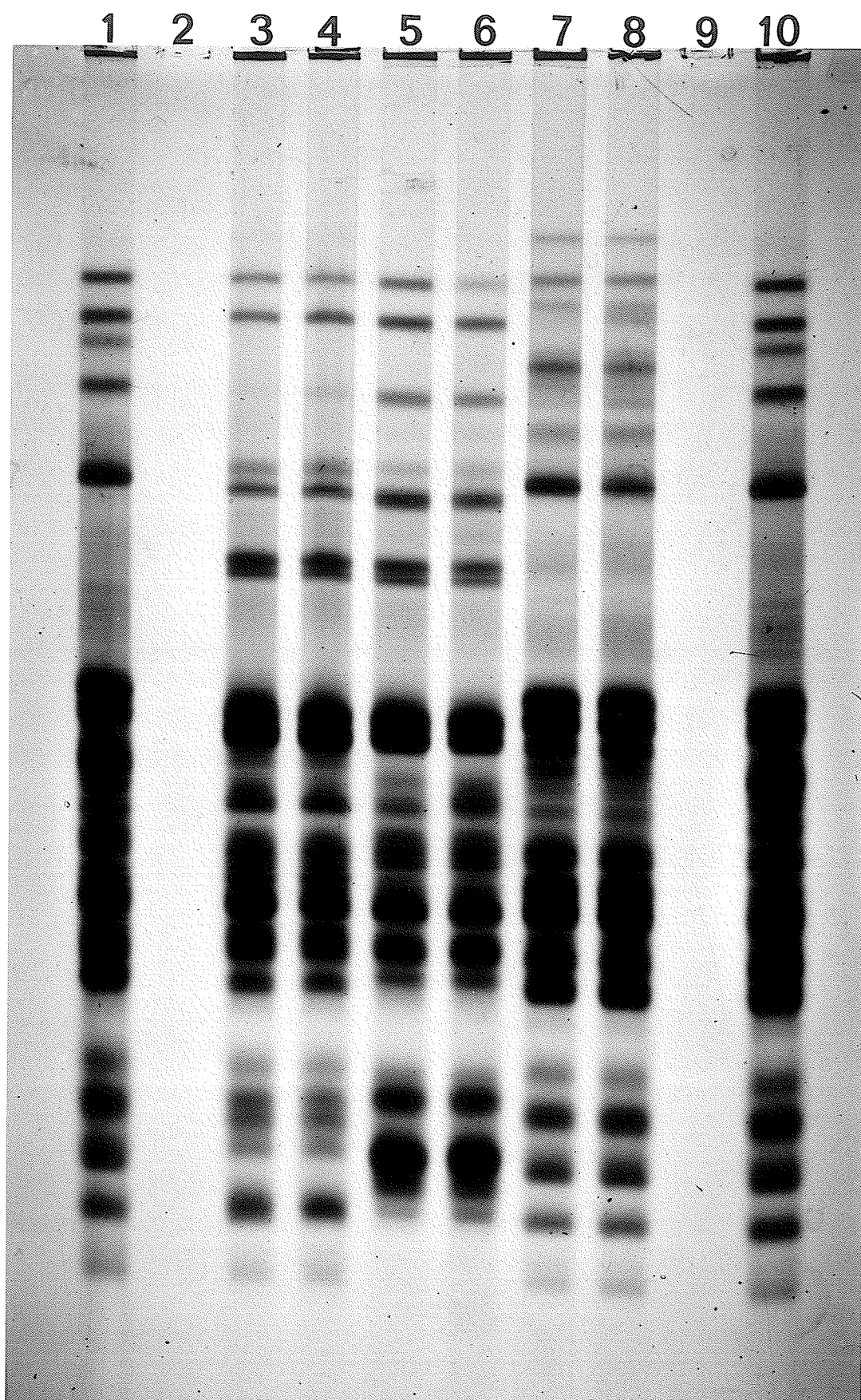


Figure 18. Gliadin electrophoregrams of wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Figure in parentheses is the protein content
(N x 5.7, 14% m.b.)

- | | | |
|----------------|--|---------------------|
| 1. Marquis | | 6. JIT-35-2L (SC) |
| | | (15%) |
| 2. ND 560 (SC) | | 7. JIT-35-2L (L) |
| (14.5%) | | (15.4%) |
| 3. ND 560 (L) | | 8. Tesopaco sib(SC) |
| (14.4%) | | (13.1%) |
| 4. Bulbul (SC) | | 9. Tesopaco sib (L) |
| (13.1%) | | (12.0%) |
| 5. Bulbul (L) | | 10. Marquis |
| (12.1%) | | |

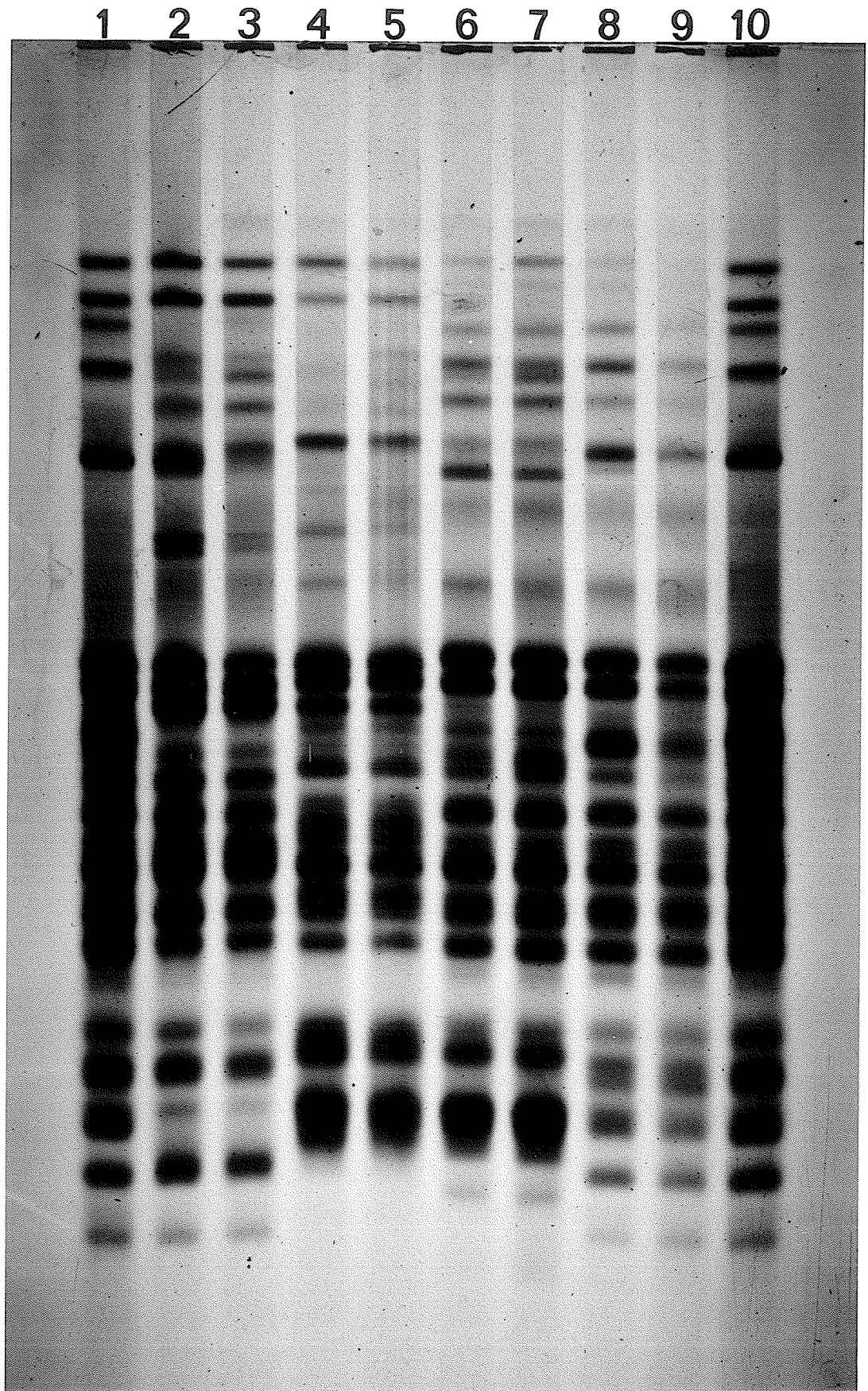


Figure 19. Gliadin electrophoregrams of wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Figure in parenthesis is the protein content
(N x 5.7, 14% m.b.)

1.	Marquis		6.	James (SD 2273)	(SC)
2.	Len (ND 543)	(SC)		(14%)	
	(14.2%)		7.	James (SD 2273)	(L)
3.	Len (ND 543)	(L)		(14.4%)	
	(14.4%)		8.	ND 563	(SC)
4.	SD 2235	(SC)		(14.9%)	
	(14.3%)		9.	ND 563	(L)
5.	SD 2235	(L)		(14.3%)	
	(14.8%)		10.	Marquis	

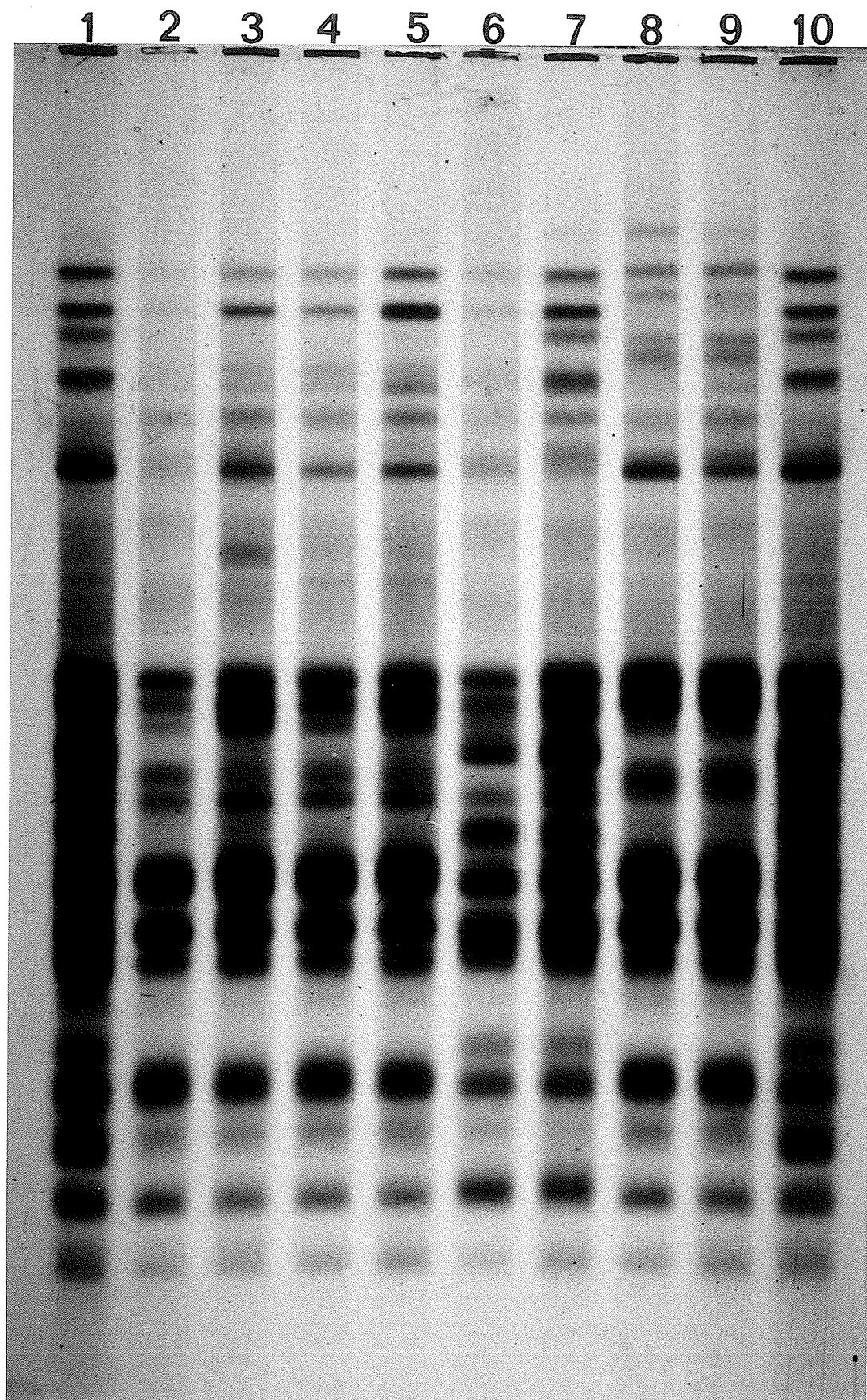
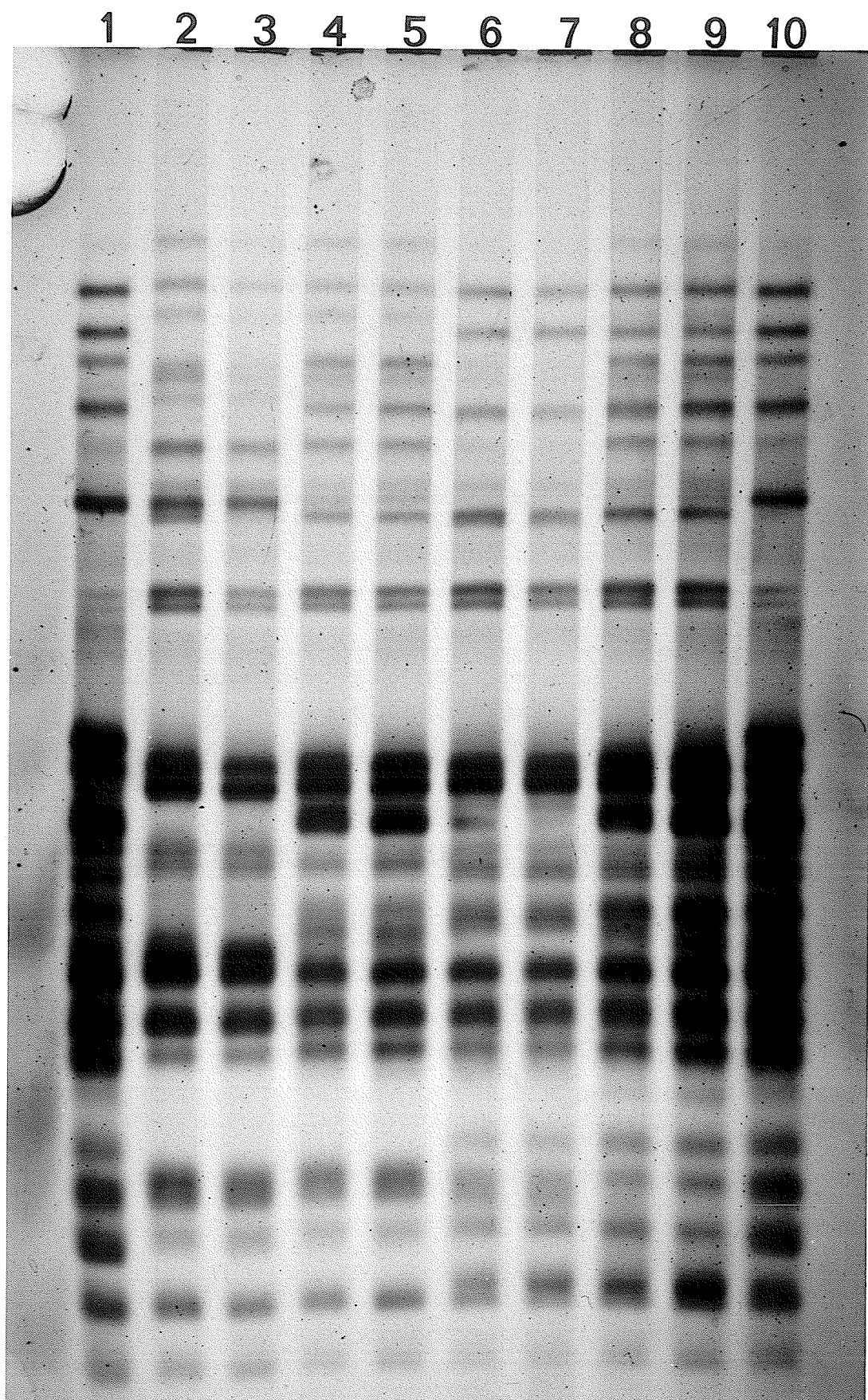


Figure 20. Gliadin electrophoregrams of wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Figure in parenthesis is the protein content
(N x 5.7, 14% m.b.)

- | | |
|-------------------------------------|------------------------------------|
| 1. Marquis | 6. NAPB NHS 183.74 (SC)
(13.2%) |
| 2. CT 790 (SC)
(14.7%) | 7. NAPB NHS 183-74 (L)
(12.5%) |
| 3. CT 790 (L)
(14.4%) | 8. Mn 70170 (SC)
(13.4%) |
| 4. NAPB NHS 1001-75 (SC)
(13.0%) | 9. Mn 70170 (L)
(12.9%) |
| 5. NAPB NHS 1001-75 (L)
(12.0%) | 10. Marquis |



APPENDIX III

SDS-PAGE Patterns of Glutenin Proteins
Isolated from Wheat Varieties Grown
at Lethbridge and Swift Current

Figure 21. SDS-PAGE patterns of glutenin proteins isolated from wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Left side: reduced glutenin
Right side: unreduced glutenin

- | | |
|---------------------------|------------------|
| 1. Mida/Cadet (SC) | 6. Chester (L) |
| 2. Mida/Cadet (L) | 7. Sinton (SC) |
| 3. Kenya 321.BT.1.B.1(SC) | 8. Sinton (L) |
| 4. Kenya 321.BT.1.B.1 (L) | 9. Sonalika (SC) |
| 5. Chester (SC) | 10. Sonalika (L) |

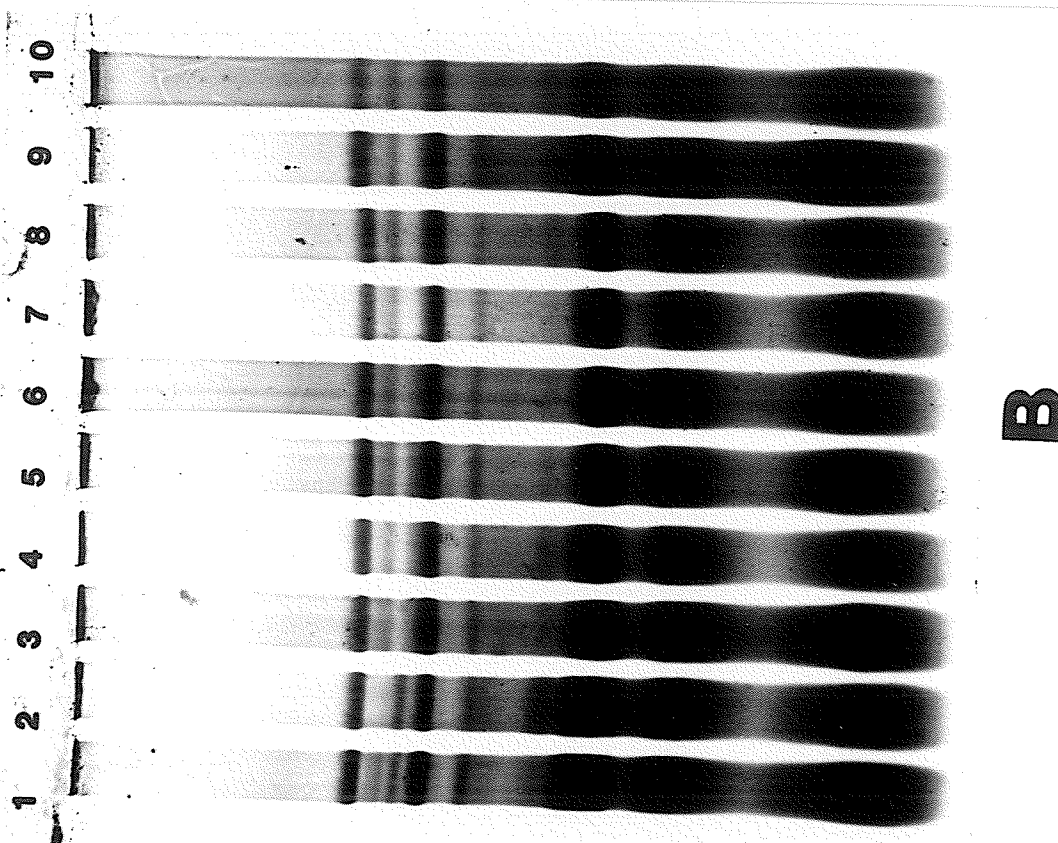


Figure 22. SDS-PAGE patterns of glutenin proteins isolated from wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Left side: reduced glutenin
Right side: unreduced glutenin

- | | | | | | |
|----|----------------------------------|------|-----|--------------|------|
| 1. | Bulbul | (SC) | 6. | JIT-35.2L | (L) |
| 2. | Bulbul | (L) | 7. | Tesopaco sib | (SC) |
| 3. | H-Ra ² F ₂ | (SC) | 8. | Tesopaco sib | (L) |
| 4. | H-Ra ² F ₂ | (L) | 9. | Pavon sib | (SC) |
| 5. | JIT-35-2L | (SC) | 10. | Pavon sib | (L) |

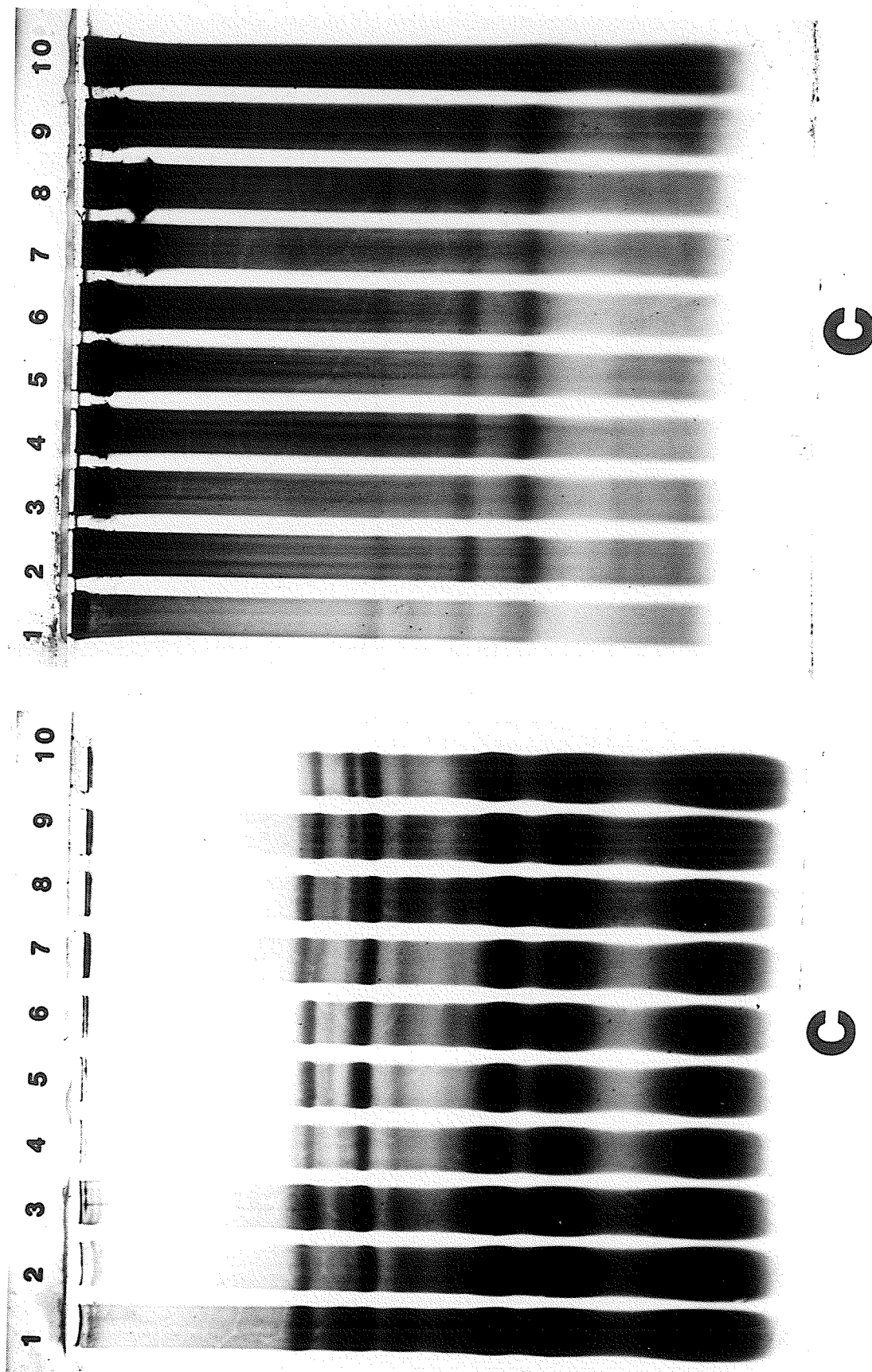


Figure 23. SDS-PAGE patterns of glutenin proteins isolated from wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Left side: reduced glutenin
Right side: unreduced glutenin

1.	SD 2235	(SC)	6.	ND 563	(L)
2.	SD 2235	(L)	7.	ND 560	(SC)
3.	James (SD 2273)	(SC)	8.	ND 560	(L)
4.	James (SD 2273)	(L)	9.	M.J. INTA	(SC)
5.	ND 563	(SC)	10.	M.J. INTA	(L)

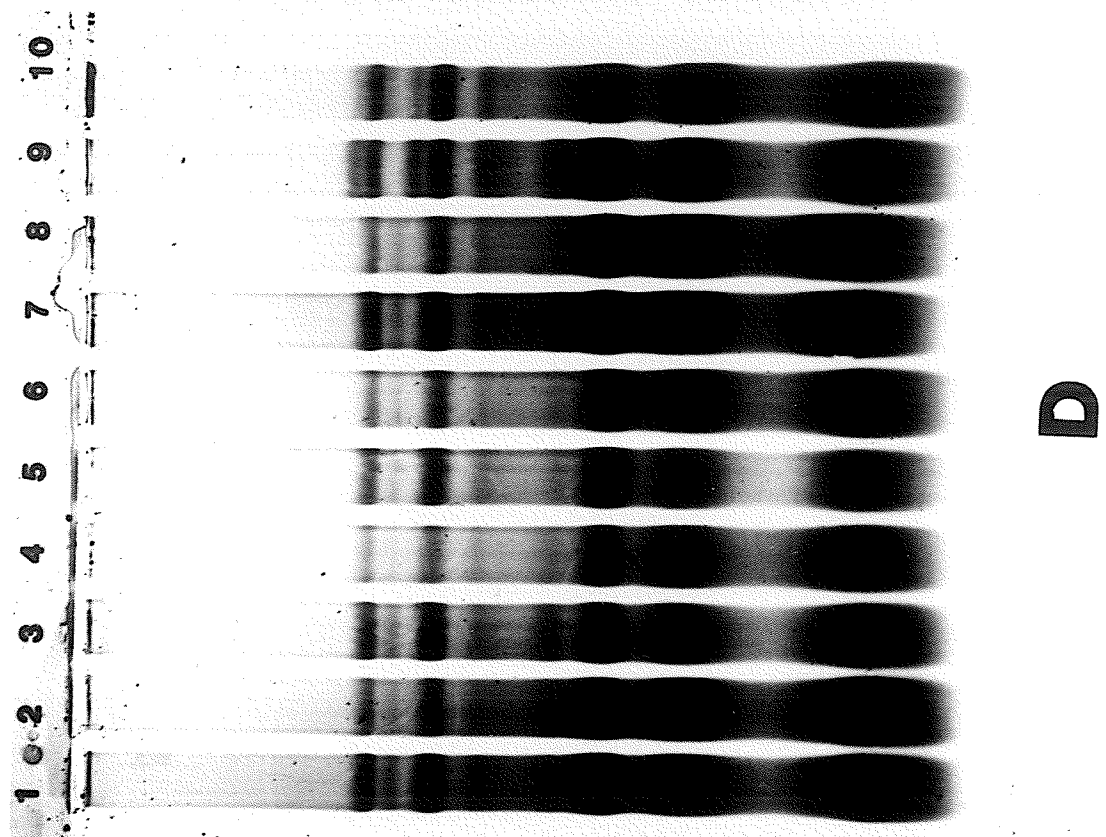
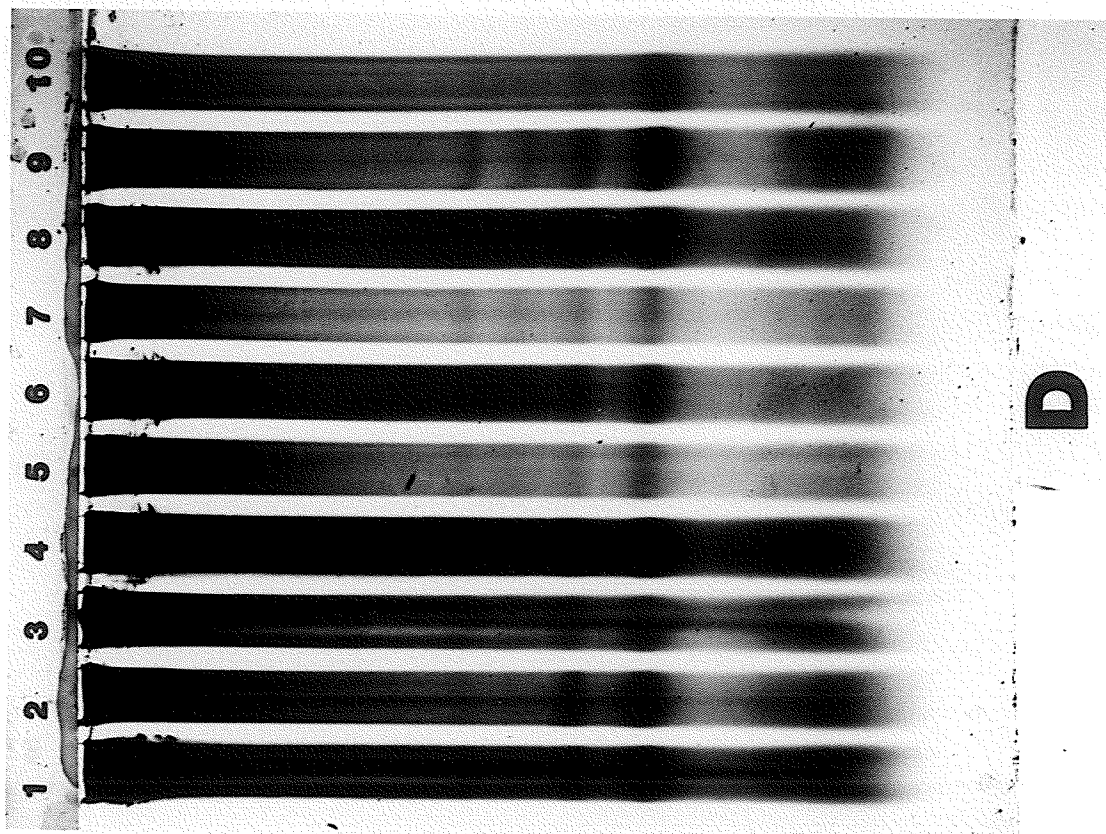
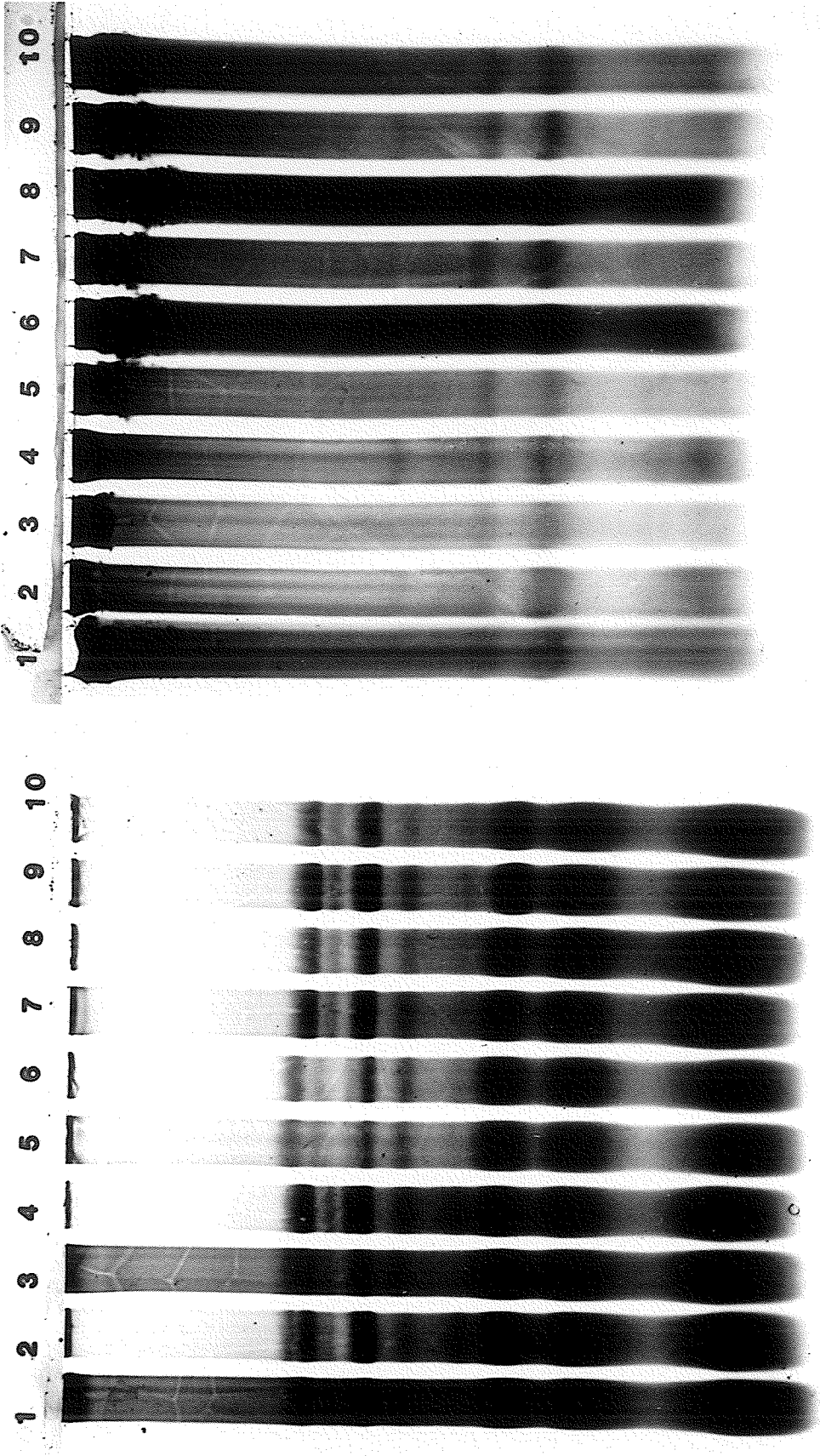


Figure 24. SDS-PAGE patterns of glutenin proteins isolated from wheat varieties grown at Lethbridge (L) and Swift Current (SC)

Left Side: reduced glutenin
Right Side: unreduced glutenin

- | | | | | | |
|----|------------------|------|-----|-----------------|------|
| 1. | CT 790 | (SC) | 2. | NAPB NHS 183-74 | (L) |
| 2. | CT 790 | (L) | 7. | Mn 70170 | (SC) |
| 3. | NAPB NHS 1001-75 | (SC) | 8. | MN 70170 | (L) |
| 4. | NAPB NHS 1001-75 | (L) | 9. | Len (ND 543) | (SC) |
| 5. | NAPB NHS 183-74 | (SC) | 10. | Len (ND 543) | (L) |



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